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INTRODUCTORY REMARKS FOR SYMPOSIUM ON THE PHYSIOLOGY OF PARASITES

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Present knowledge concerning the metabolic requirements of endoparasites has been accumulated almost exclusively during the 20th century, since only a very few papers were published previously. The early work of Claude Bernard (1859) and Foster (1865) demonstrated the occurrence of glycogen in helminths but had little influence on future developments in the field. A more important contribution was the finding of Bunge (1889) that *Ascaris* could be kept alive for several days outside the host in the absence of oxygen, and that during this time it produced carbon dioxide and a volatile acid. This work furnished the stimulus for Weinland's (1901 to 1904) classical series of papers in which he demonstrated that *Ascaris*, in the absence of oxygen, gains energy through fatty acid fermentation of glycogen.

Weinland was of the opinion that the intestinal helminths are purely anaerobic animals, and his views dominated the field for the next 30 years. However, Alt and Tischer (1931) and Adam (1932) demonstrated that *Moniezia* and *Ascaris* are actually capable of using oxygen, if it is supplied to them. Thus no general answer can be given to the question as to whether helminths gain their energy primarily through oxidations or fermentations. Rather, evidence indicates that the mechanism may vary with the species.

The early work on helminths was confined largely to the identification of the overall metabolic processes in such large, easily obtainable worms as *Ascaris*. More recently, interest has shifted toward studies on intermediate processes with emphasis on enzymatic relationships and more attention has been given to other species.

Present knowledge of the metabolic relationships of helminths has been derived mainly from short-time experiments *in vitro*. A modicum of success has been attained in a few instances in the cultivation of helminth parasites but such studies have added little to our knowledge of the physiology of such forms. Undoubtedly more work needs to be done in this field. Greater rewards will redound from a more critical and more fundamental approach to the problem and will pave the way for further elucidation of the physiology of these parasites. Such has already occurred

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in connection with the cultivation of the parasitic protozoa. The studies of Lwoff (1940) on members of the Trypanosomidae, of Cailleau (1937) on trichomonads, of Rees et al. (as reported by Rees and Reardon, 1947) on *Endamoeba histolytica*, and of McKee et al. (1946) on *Plasmodium* have provided important clues to the growth requirements of these forms.

Since successful metabolic studies on protozoa can be carried out only with species obtainable without bacterial contaminants, such investigations have been confined largely to the trypanosomes and the malarial parasites. Historically, the trypanosomes were the first of the parasitic protozoa to be employed successfully for physiological research, the initial important contributions being made by von Fenyvessy and Reiner (1924) and by Yorke, Adams, and Murgatroyd (1929). The first direct investigation of the metabolism of a malarial parasite was carried out by Christophers and Fulton (1938). The advent of the war added impetus to the interest in this field and since that time several groups of workers have made very important contributions.

This brief historical review will serve to indicate the limited extent of our knowledge in this field. The science of parasitology, like other sciences, has over the years gone through many different cycles and many changing trends. Unlike bacteriology, in which marked advances have been made in recent years in the biochemistry and physiology of bacteria, parasitology is far behind in this vital phase. Somewhat blindly, we have been endeavoring to learn something of host-parasite relationships, chemotherapy, immunology, and of the manner in which parasites damage their hosts without an adequate understanding of their physiological requirements and the method by which they carry on their metabolism in the varying types of environment in which they exist within the bodies of their intermediate and definitive hosts. We need more parasitologists interested in physiology and biochemistry and more physiologists and biochemists interested in parasitology. Furthermore, what is clearly indicated is better and more extensive training for parasitologists in these collateral fields.

It is hoped that this symposium will bring to you information on latest developments in this field; that it will indicate also the very limited breadth of our knowledge; that it will point out the importance of problems still awaiting solution; and that it will stimulate your interest in helping to solve these problems.

The speakers this morning are recognized authorities in the field. I have leaned heavily on each of them for advice in the organization of this program and am particularly indebted to Dr. Theodor von Brand for his assistance.

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THE CARBOHYDRATE METABOLISM OF PARASITES

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Most hitherto studied endoparasites have a very pronounced carbohydrate metabolism. The reasons for this fact seem to be different in various cases. The parasites living in anaerobic or semi-anaerobic habitats gain their energy almost exclusively through the fermentation of carbohydrates because it is a much better substratum for gaining anaerobic energy than either protein or fat. Hellerman (1947) has pointed out that the occurrence of intermediately oxidized groupings

($\text{H}-\overset{\textstyle |}{\underset{\textstyle |}{\text{C}}}-\text{OH}$) in the hexose molecule endows it "with characteristic sensitivity in conditions that induce essentially intramolecular oxidation-reductions or rearrangements." Examples of parasites forced by lack of oxygen to a type of metabolism characterized by the preponderance of carbohydrate consumption are the large helminths of the intestinal lumen.

Parasites having access to oxygen in one way or the other, by virtue of their location within the host, e.g. the swim bladder of fish, or by virtue of their feeding habits (bloodsucking, Wells, 1931), may have a less pronounced need for carbohydrates, but little definite information is available. The fact that some small nematode species (*Nematodirus*, *Nippostrongylus* and *Neoaplectana*, Rogers, 1948) have a relatively low respiratory quotient is suggestive in this connection but not a proof, since low respiratory quotients can occur under a variety of conditions. Theoretically, these small worms may gain significant amounts of oxygen in surroundings forcing larger species to an almost purely anaerobic life (von Brand, 1938b) and may thus be able to metabolize other substances besides carbohydrate.

It is, however, impossible to generalize. The biochemical constitution of organisms is just as important as external factors in determining their metabolism. This is well known for free-living invertebrates. A honeybee, for example, is very dependent on an adequate source of carbohydrate (Beutler, 1936); leeches metabolize chiefly proteins (Braconnier-Fayemendy, 1933); and so on. Similarly, there is no difficulty in finding parasites occurring in oxygen-rich surroundings that metabolize chiefly carbohydrates. The schistosomes (Bueding and Oliver-Gonzalez, 1948), or the bloodstream form of the African trypanosomes (von Brand and Tobie, 1948) may be mentioned in this connection. These organisms as well as almost all other parasitic protozoa and worms, are aerobic fermenters; that is, they do not completely oxidize the carbohydrate, but decompose it only partially. The only exception so far found is *Plasmodium lophurae* (Wendel, 1946).

The biological significance of this lack of complete oxidation is not known, and its mechanisms are but insufficiently understood. A lack of the requisite enzyme systems is certainly involved at least in some cases, as the African trypanosomes. In other cases, for example the malarial parasites, not so much a lack of enzyme

systems, but rather a differential activity of different systems seems to be important. McKee, Ormsby, Anfinson, Geiman, and Ball (1946) found the lactic acid production of *Plasmodium knowlesi* to proceed at a much faster rate than its further utilization.

In the case of helminths the question is tied up with that of the significance of aerobic processes. Most roundworms can probably utilize interchangeably the energy derived from anaerobic processes and that from oxidations, as indicated by the occurrence of a more or less pronounced Pasteur effect in such forms as *Eustrongylides* (von Brand and Simpson, 1945) or *Litomosoides* (Bueding, 1948, 1949a). In these forms the situation may parallel that found in malarial parasites. In flat worms, such as the schistosomes, and in the roundworm *Dracunculus insignis* no Pasteur effect has been found (Bueding, Peters, and Welch, 1947; Bueding, 1949c) and the possibility, but not yet certainty, exists that in these forms the oxidative carbohydrate metabolism is a rudimentary function which cannot substitute for anaerobic processes (Bueding, 1949b). The persistence of the latter under aerobic conditions could be easily understood on this basis.

The question of the factors responsible for the persistence of aerobic fermentations should be investigated further. It is of considerable theoretical interest and may be also of practical importance. The pathogenicity of some parasites at least may be correlated with this failure of complete oxidation (though not necessarily of carbohydrate alone) which has the obvious consequence that the partially oxidized metabolites come in contact with the host tissues either directly after excretion, as in the case of the blood and tissue parasites, or after absorption, as in the case of intestinal parasites.

Sources of carbohydrate

All carbohydrate used by parasites is of course ultimately derived from material provided by the host and therefore from the parasite's standpoint exogenous. However, some parasitic forms depend on a constant supply of such exogenous carbohydrate, while others do not. The best example of the former group is the blood-stream form of the African trypanosomes. These organisms die very rapidly if their surroundings become depleted of sugar which they find in sufficient concentration under natural conditions in the blood or in the spinal fluid (von Brand, 1933b). Among the worms, the filariae and schistosomes may belong to this group, since they have a pronounced carbohydrate metabolism but store only relatively little polysaccharide within their tissues (Bueding and Oliver-Gonzalez, 1948; Bueding, 1949a; Axmann, 1947).

The importance of exogenous carbohydrate has become evident also from a study of the influence of the host's diet on the parasites. Hegner and Eskridge (1937) described a favorable influence of a carbohydrate-rich diet on the development of amoebae in rats; Westphal (1939) found *Chilomastix* and *Enteromonas* to increase suddenly in numbers as soon as starch became available in their natural habitat, and Armer (1944) observed that a high carbohydrate diet definitely favored the development and persistence of several intestinal protozoa of the cockroach. As to metazoan parasites, it has been proven conclusively that *Hymenolepis diminuta* depends on an adequate supply of carbohydrate in the host's diet (Chandler, 1943) and the same appears to be true for *Railletina cesticillus* (Reid, 1942).

Many, but not all, parasites have an endogenous source of carbohydrate on which they can draw when no exogenous carbohydrate is available. The most common reserve carbohydrate is glycogen. Morphological studies have revealed large stores of polysaccharide in gregarines (Buetschli, 1885), coccidia (Giovannola, 1934, Edgar, Herrick and Fraser, 1944), trichomonads (Stewart, 1938), in many parasitic ciliates (Westphal, 1934; Hungate, 1943a; Armer, 1944), and in mesozoa (Nouvel, 1929, 1931). As to endoparasitic worms and arthropods, the quantitative data assembled in table 1 prove that many forms have large stores of glycogen indeed.

A survey of the data of table 1 makes it apparent that parasites living in surroundings with low oxygen content, or in surroundings in which periodically severe oxygen deficiencies may occur (e.g., stomach) have usually large polysaccharide reserves. Exceptions are *Ancylostoma caninum* and adult *Trichinella spiralis* (Oliver-Gonzalez and Bueding, 1948). Both, however, in nature may not lead a primarily anaerobic life, the former getting oxygen from the host's blood, the latter by virtue of its intimate contact with the host's intestinal mucosa.

Less regularity exists in the case of tissue parasites which all live in habitats with at least moderately high oxygen tension. Some, as *Cysticercus fasciolaris* or *Eustrongylides* larvae, have exceedingly high polysaccharide reserves; others, like the filarial worms or the schistosomes, accumulate only small stores, and in the bloodstream form of the trypanosomes (Krijgsman, 1936; Moulder, 1948b) or the microfilariae of *Wuchereria bancrofti* (Brault and Loeper, 1904) no polysaccharide whatever has been found.

The glycogen reserves of the parasites seem to be universally derived from carbohydrates ingested as food. So far, no convincing evidence has been presented that protein or fat may serve as mother substance. Ingested starch, as in the case of *Endamoeba histolytica* in culture, or cellulose, as in some rumen ciliates (Weineck, 1934, Hungate, 1943a) and termite flagellates (Cleveland, 1924; Hungate, 1943b), may be used for the synthesis of glycogen or par glycogen, after having undergone preliminary digestion.

In worms, polysaccharide synthesis is largely dependent upon the availability of lower carbohydrates, especially glucose. A glycogen-sparing effect, or a slight increase in glycogen content has been observed in several nematodes and cestodes upon the addition of glucose to the medium (Weinland and Ritter, 1902; von Brand, 1933a; Wardle, 1937; Markov, 1939). A very intensive rate of glycogen synthesis has been observed by Bueding (1949a) in *Litomosoides carinii* in which the glycogen content increased by about 100 percent during 3 hours incubation in a medium containing 0.02 m. glucose.

Very little is known so far concerning the mechanism of polysaccharide formation, no data whatever being available for parasitic protozoa or arthropods. In water extracts of *Ascaridia galli* incubated in a medium containing 0.005 m. NaF, adenylic acid, and glucose-1-phosphate, Rogers and Lazarus (1949) found a definite polysaccharide synthesis from the Cori ester. A low grade synthesis was also observed in fluoride-treated muscle brei of *Ascaris lumbricoides* without addition of glucose-1-phosphate to the system. On the whole it appears probable that the mechanism of glycogen synthesis is the same as that occurring in vertebrates.

In most cases the glycogen deposits of parasites unquestionably represent an energy reserve, but may serve also other purposes. It is reasonable to assume that

in parasitic arthropods glycogen serves also as a reservoir for the carbohydrate required for chitin synthesis. This function has been well established in the case of crustaceans (Verne, 1924). The same assumption is justified for those nematodes and acanthocephala that produce chitinous egg membranes (Chitwood, 1938; Jacobs and Jones, 1939; von Brand, 1940). In ascarids about half the glycogen stored in the oocytes serves to form the glucosamine incorporated in the chitin of the egg membrane (Fauré-Fremiet, 1913; Szejewska, 1929). In other instances the

TABLE 1.—Glycogen content of parasitic worms and arthropods

Species	Glycogen in percent of		Habitat	Availability of significant amounts of O ₂	Author
	fresh weight	dry weight			
Trematodes					
<i>Fasciola hepatica</i>	3.1 to 3.7	15 to 21	Bile ducts	No	Flury and Leeb, 1926; Weinland and von Brand, 1926
<i>Schistosoma mansoni</i> ♂		14 to 26	Bloodstream	Yes	Bueding personal communication
<i>Schistosoma mansoni</i> ♀		2 to 5	"	"	Bueding personal communication
Cestodes					
<i>Moniezia expansa</i>	2.7 to 3.2	24 to 32	Intestine	No	Weinland, 1901a; von Brand, 1933a; Wardle, 1937
<i>Moniezia denticulata</i>	1.6	19	"	"	von Brand, 1933a
<i>Taenia saginata</i>	7.4	60	"	"	Smorodincev and Bebesin, 1936
<i>Taenia solium</i>	2.2	25	"	"	Smorodincev and Bebesin, 1936
<i>Taenia marginata</i>	6.7	28	"	"	von Brand, 1933a
<i>Taenia plicata</i>	1.6	6	"	"	von Brand, 1933a
<i>Diphyllobothrium latum</i>	1.9	20	"	"	Smorodincev and Bebesin, 1936
<i>Railletina cesticillus</i>	6.5	32	"	"	Reid, 1942
<i>Triacnophorus nodulosus</i>		14	"	?	Markov, 1939
<i>Eubothrium rugosum</i>		23	"	?	Markov, 1939
<i>Cysticercus fasciolaris</i>		28	Liver	?	Salisbury and Anderson, 1939
<i>Ligula intestinalis</i>		34	Body cavity	?	Markov, 1939
Nematodes					
<i>Ascaris lumbricoides</i>	5.3 to 8.7	24 to 44	Intestine	No	Weinland, 1901a; Flury, 1912; Smorodincev and Bebesin, 1936; von Brand, 1937
<i>Parascaris equorum</i>	2.1 to 3.8	10 to 23	"	"	Schimmelpfennig, 1903; Toryu, 1933
<i>Ascaridia galli</i>	3.6 to 4.7		"	?	Reid, 1945a, b
<i>Ancylostoma caninum</i>	1.6		"	Yes	von Brand and Otto, 1938
<i>Strongylus vulgaris</i>	3.5		"	?	Toryu, 1933
<i>Eustrongylides ignotus</i> (larvae)	6.9	28	Various organs	Yes	von Brand, 1938a
<i>Litomosoides carinii</i>	0.8		Pleural cavity	"	Bueding, 1949a
<i>Dirofilaria immitis</i>	1.9	10	Heart	"	von Brand, unpublished
<i>Dipetalonema gracilis</i>	0.2		Abdominal cavity	"	von Brand, unpublished
Acanthocephala					
<i>Macracanthorhynchus hirudinaceus</i>	1.1 to 2.3	8 to 13	Intestine	No	von Brand, 1939, 1940
Arthropods					
<i>Gasterophilus intestinalis</i> (larvae)	5.0 to 8.6	14 to 31	Stomach	Variable	von Kemnitz, 1916

significance of glycogen is none too obvious. A case in point is the parasitic amoebae. While the evidence indicates rather clearly that the glycogen vacuole of *Iodamoeba* is actually a food store (von Brand, 1932), the significance of the rapid appearance and equally rapid disappearance of a large glycogen vacuole in the young *Endamoeba* cysts is an unsolved riddle and its elucidation a challenging problem.

Quantitative aspects of carbohydrate metabolism

Several parasites have a surprisingly high rate of carbohydrate metabolism. Among protozoa, the highest rates have been found in the bloodstream forms of

the African pathogenic trypanosomes (Christophers and Fulton, 1938; von Brand and Tobie, 1948) where the hourly glucose consumption amounts to about 50 to 100 percent of the dry weight. Among helminths, the highest rate has been observed in schistosomes with an hourly rate of about 20 percent of their dry weight (Bueding, Peters, and Waite, 1947; Bueding and Oliver-Gonzales, 1948). Data suggesting a high rate of carbohydrate consumption have also been presented for various parasitic protozoa, such as malarial parasites (Wendel, 1943; Silverman, Ceithaml, Taliaferro, and Evans, 1944; McKee, Ormsbee, Anfinson, Geiman, and Ball, 1946), trichomonads (Andrews and von Brand, 1938), rumen ciliates (Westphal, 1934), and termite flagellates (Hungate, 1943b). Due to technical difficulties, however, these data cannot as yet be correlated to either the fresh or dry weight of the organisms. It would be erroneous, however, to assume that all parasitic protozoa are characterized by a high rate of carbohydrate consumption. It has thus not been pos-

TABLE 2.—Carbohydrate consumption of parasites in gm. per 100 gm. in 24 hours at 37 to 41° C

Species	Glycogen consumption		Author
	Anaerobic	Aerobic	
Trematodes			
<i>Fasciola hepatica</i>	2.6		Weinland and von Brand, 1926
<i>Schistosoma mansoni</i> *	79 to 96	79 to 96	Bueding (personal communication)
Cestodes			
<i>Moniezia expansa</i>	1.0		von Brand, 1933a
<i>Railletina cesticillus</i>	4.8		Reid, 1942
Nematodes			
<i>Ascaris lumbricoides</i>	1.4	1.2	von Brand, 1934
<i>Parascaris equorum</i>	1.4	1.6	Toryu, 1936
<i>Ascaridia galli</i>	3.6		Reid, 1945b
<i>Eustrongylides ignotus</i> (larvae)	0.7	0.2	von Brand and Simpson, 1945
<i>Litomosoides carinii</i> *	34 to 41	42 to 45	Bueding (personal communication)
Acanthocephala			
<i>Macracanthorhynchus hirudinaceus</i>	1.0	0.8	Rudolph (fide Weinland, 1910) ; von Brand, 1940
Arthropods			
<i>Gasterophilus intestinalis</i> (larvae)	0.7	1.3	von Kemnitz, 1916

* Total carbohydrate consumption in nutritive, sugar containing media; all other figures for starving parasites.

sible to demonstrate, by means of morphological methods, a polysaccharide decrease in gregarines starving for a long time within the intestine of mealworms (von Brand, unpublished), or, by means of quantitative determinations, a glucose consumption by the bloodstream form of *Trypanosoma cruzi* (von Brand, Tobie, Kissling, and Adams, 1949).

Several quantitative data for the glycogen consumption of helminths are available, but only a single one for a parasitic arthropod (table 2). Most of these determinations have been done with worms starving *in vitro* in more or less unphysiological media like salines of different composition. This raises the question whether or not these values correspond to those occurring under natural conditions. While obviously no unequivocal answer can be given, the present author is on the whole inclined to answer it affirmatively. Weinland (1901b) observed an identical glycogen consumption of ascarids starving *in vitro* and *in vivo* as did Reid (1945b) in the case of *Ascaridia galli*, and at least one of the worms studied, *Eustrongylides*, survives for such long periods *in vitro* (von Brand and Simpson, 1944) that short time experiments under somewhat unphysiological conditions should not be objectionable.

The data of table 2 show that the glycogen consumption rates of starving

helminths are influenced only to a small degree by the presence or absence of oxygen. This is in marked contrast to what has been found in most free-living invertebrates (summary in von Brand, 1946; newer data in Cleary, 1948) in which the anaerobic rate is almost invariably much higher than the aerobic one. Free-living invertebrates as a rule oxidize the carbohydrate completely in the presence of sufficient oxygen thus gaining a maximum of energy from a minimum of substance, while under anaerobic conditions a maximum of carbohydrate has to be mobilized in order to liberate the minimum of energy required for the sustenance of life. In parasitic worms, on the contrary, aerobic fermentations prevail to a varying degree even if they are kept under high oxygen tensions. This drives the rate of aerobic carbohydrate consumption up, in most cases very close to the anaerobic rate. It is quite possible, however, that future research may reveal parasites behaving like free-living organisms in this respect; they would probably have to be looked for among forms occurring in oxygen rich habitats. A suggestion in this direction is *Eustrongylides* which consumes anaerobically about 3 times as much glycogen as aerobically. The only organism consuming less glycogen under anaerobic conditions than in the presence of air is the *Gasterophilus* larva. The reasons for this aberrant behavior are not quite clear; its anaerobic energy requirements must be very much smaller than its aerobic needs.

The data of table 2 illustrate strikingly another fact, namely, the great differences in the rates of carbohydrate consumption of various helminths. Among the cestodes, *Railletina cesticillus* consumes about 5 times as much glycogen as *Moniezia expansa*, and among the nematodes, *Litomosoides carinii* about 50 to 80 times as much carbohydrate as *Eustrongylides ignotus*.

It is probable that no single factor explains these differences. The size of the organisms will be one factor, small organisms having quite generally a higher metabolic rate than large ones. Various degrees of motility may be important, the influence of muscular movement on metabolism requiring no emphasis. Various types of aerobic and anaerobic fermentations occur in different species and it can be expected that their energy release will be different. Finally, the nutritional state has to be considered. *Schistosoma mansoni* and *Litomosoides carinii* obviously cannot satisfy their enormous carbohydrate requirements from an endogenous source alone, even during relatively short experimental periods; the values listed in table 2 are therefore derived from experiments in which the worms had an exogenous source of carbohydrate. The *Eustrongylides* larvae, on the contrary, show, at least under aerobic conditions, an approximately equal rate of carbohydrate consumption when they are starving or are kept in a sugar containing medium.

Endproducts of aerobic and anaerobic fermentations

Vertebrate tissues exposed to lack of oxygen break down carbohydrate mainly to lactic acid. In yeasts and bacteria, on the other hand, a great variety of different endproducts is formed. Parasites seem, insofar as the diversity of endproducts is concerned, to be about intermediate between the above groups.

Of gaseous endproducts, hydrogen has been identified only in the case of termite flagellates (Hungate, 1943b); it may also be produced by *Trichomonas foetus* (Andrews and von Brand, 1938), but apparently not by metazoan parasites.

Carbon dioxide found in anaerobic experiments can be of different origin. It can be "inorganic" carbon dioxide liberated from bicarbonate through stronger acids,

or it can be true respiratory CO_2 ; that is, its carbon can be derived from the metabolized carbohydrate. The question of carbon dioxide retention in the tissues and the medium has also to be considered. Bueding (1949b) has pointed out that in view of this complicated situation a correct evaluation of most of the data available for anaerobically kept helminths is not possible. The problem of whether or not CO_2 is an endproduct of anaerobic carbohydrate utilization is important and the question arises whether or not it can be answered at least qualitatively.

The formation of fatty acids especially would be difficult to understand without concomitant formation of CO_2 from the carbohydrate molecule. It has been shown conclusively that true respiratory CO_2 is formed by the *Trichinella larvae* (Stannard, McCoy and Latchford, 1938) which, however, do not produce organic acids, and during the anaerobic dismutation of pyruvic acid to acetic and lactic acid by *Litomosoides* (Bueding, 1949a). In the case of *Ascaris*, von Brand's (1934) data show that the excreted acids could account maximally for only 15 percent of the actually eliminated CO_2 . In von Kemnitz' (1916) experiments with *Gasterophilus* the bicarbonate content of the larvae should have amounted to at least 0.9 percent of the fresh tissue weight if all the excreted CO_2 were of inorganic origin, obviously an impossible value. It seems justified therefore to assume in these and similar cases that the greater part of the evolved CO_2 was of organic origin. True quantitative figures, however, will be very difficult to secure with large worms like *Ascaris* or with tape worms containing calcareous corpuscles.

The main organic endproducts resulting from fermentative carbohydrate utilization by several parasitic protozoa, worms, and arthropods are listed in tables 3 and 4. It is apparent that pyruvic acid and lactic acid are the most common endproducts of the protozoa thus far studied, although lactic acid is definitely not produced by some trypanosomes. The only parasites in which lactic acid is the only, or at least quantitatively the most important, endproduct of anaerobic carbohydrate utilization are the malarial parasites, the schistosomes, and the filariid worms. They resemble vertebrate tissues in this respect. It is noteworthy that *Schistosoma mansoni* produces (D,L)-lactic acid, while *Litomosoides carinii* excretes L(+)-lactic acid (Bueding, 1949b).

In ascarids volatile fatty acids predominate, valeric acid being the most important one, while in *Fasciola*, *Moniezia*, and the arthropod *Gasterophilus* higher fatty acids are characteristic. It is true that the occurrence of fatty acid fermentations has been doubted (Fischer, 1924; Slater, 1928) but the more recent reviewers (Smyth, 1947; Hobson, 1948; Bueding, 1949b) consider the balance of evidence as favorable to the concept of causal connection between carbohydrate breakdown and fatty acid production. It does not appear necessary to review the various arguments at this occasion; irrefutable proof one way or the other will come either from the use of worms grown under aseptic conditions or from the use of sterile extracts.

The question whether or not the endproducts of anaerobic and aerobic fermentations of a given species are qualitatively identical cannot be answered definitely in every case. In ascarids, for instance, the differences implied by the data shown in table 4 may be real or, on the other hand, may be due to the different techniques of identification used by various investigators. A comparative study with identical methods has not yet been carried out. In other cases definite qualitative differences do occur. Thus glycerol accumulates only in anaerobic incubates of *Trypanosoma*

TABLE 3.—Organic endproducts of aerobic and anaerobic fermentations of protozoa

Species	Condition	Pyruvic acid	Lactic acid	Formic acid	Acetic acid	Oxalic acid	Succinic acid	Ethyl alcohol	Glycerol	Author
Termite flagellates	Anaerobic				x					Hungate, 1939 ; 1943b
<i>Leishmania brasiliensis</i> , <i>L. donovani</i> , <i>L. tropica</i> (Culture form)	Aerobic	x	x	x			x			Chang, 1948
<i>Trypanosoma lewisi</i> (Bloodstream form)	Aerobic				x	x	x	x		Reiner, Smythe, and Pedlow, 1936
	Anaerobic				x		x			
<i>Trypanosoma cruzi</i> (Culture form)	Aerobic	x	x	x			x			Chang, 1948
<i>Trypanosoma equiperdum</i> , <i>T. hippicum</i> (Bloodstream form)	Aerobic	x								Reiner, Smythe, and Pedlow, 1936 ;
	Anaerobic	x						x		Harvey, 1949
<i>Trypanosoma brucei</i> (Bloodstream form)	Aerobic	x				x				Glowazky, 1937
	Anaerobic	x	x			x		x		
<i>Trypanosoma rhodesiense</i> (Bloodstream form)	Aerobic	x	x	x	x		x	x	x	Fulton and Stevens, 1945
<i>Plasmodium gallinaceum</i>	Aerobic	x	x		x		x			Silverman, Celthaml, Tallafarro, and
	Anaerobic	x	x							Evans, 1944 ; Speck, Moulder, and
										Evans, 1946
<i>Plasmodium knowlesi</i>	Aerobic	x	x							Wendel, 1943 ; McKee, Ormsbee, Anfinson, Geiman, and Ball, 1946

equiperdum or *T. hippicum*, because it is readily oxidized further in the presence of oxygen. Among helminths definite differences also occur. *Litomosoides carinii* forms acetylmethylcarbinol, in addition to the organic acids listed in table 4, only under aerobic conditions (Berl and Bueding, *vide* Bueding, 1949b). Quantitative differences in the proportions of the various endproducts may occur frequently. One of the best examples is *Litomosoides* where the lactic acid/acetic ratio is quite different under aerobic and anaerobic conditions (Bueding, 1949a).

TABLE 4.—Organic acids produced by worms and arthropods

Species	Condition	Lactic acid	Formic acid	Acetic acid	Propionic acid	Butyric acid	Valeric acid	Caproic acid	Higher fatty acids	Succinic acid	Author
Trematodes											
<i>Fasciola hepatica</i>	Anaerobic					x			x		Flury and Leeb, 1926 ; Weinland and von Brand, 1926 ; Stephenson, 1947
<i>Schistosoma mansoni</i>	Aerobic	x									Bueding, Peters, and Waite, 1947
	Anaerobic	x									
Cestodes											
<i>Moniezia capansa</i>	Aerobic	x							x		Alt and Tischer, 1931 ; von Brand, 1933a
	Anaerobic	x							x	x	
Nematodes											
<i>Ascaris lumbricoides</i>	Aerobic	x	x	x			x				Weinland, 1904 ; Flury, 1912 ; von Brand, 1934 ; Oesterlin, 1937
	Anaerobic	x	x			x	x	x			
<i>Parascaris equorum</i>	Aerobic	x			x						Fischer, 1924 ; Toryu, 1936
	Anaerobic	x			x		x				
<i>Litomosoides carinii</i>	Aerobic	x			x						Bueding, 1949a
	Anaerobic	x		x							
<i>Dracunculus insignis</i>	Aerobic	x									Bueding, 1949c
	Anaerobic	x									
Arthropods											
<i>Gasterophilus intestinalis</i> (larvae)	Anaerobic	x							x		von Kemnitz, 1916 ; Blanchard and Dinulescu, 1932

Intermediate carbohydrate metabolism

In aerobic organisms the carbohydrate breakdown is conventionally divided into two parts. The first leads to pyruvic and lactic acid and requires no oxygen; it can therefore proceed also in anaerobic organisms. The second part involves the utilization of oxygen and leads ultimately to carbon dioxide and water.

The anaerobic phase is known as the Embden-Meyerhof scheme and comprises a series of reactions mediated by specific enzymes. Evidence for the presence or absence of such a chain of reactions can be gathered in various ways: The organisms, or the medium in which they are kept, can be analyzed for the presence of intermediates, for example phosphorylated compounds; it can be studied whether or not the organisms or extracts therefrom are capable of transforming one intermediate into another; or their reactions to inhibitors known to interfere with certain steps of the chain can be studied.

These various methods have been applied only in very recent years to parasites and the available evidence has been assembled in table 5. It is quite evident that the anaerobic sugar breakdown in those trypanosomes and malarial parasites that have been studied follows essentially the same pattern as found in the tissues of many free-living organisms. Certain differences do occur. In trypanosomes of the *evansi* group the degradation process stops at the pyruvate stage and glycerol is formed, probably from one of the triose compounds. Whether or not the glycolytic enzymes of these parasites are in every respect similar to those of vertebrate tissues remains to be studied.

Less detailed work has been done on helminths and none whatever on endoparasitic arthropods. Only two typical enzymes of the glycolytic chain have thus far been identified in the case of *Schistosoma mansoni*. It is very likely that phosphorylations also play an important role in parasitic worms. It has been shown that *Litomosoides carinii* incorporates more inorganic radioactive phosphorus into organic acid soluble phosphate in the presence than in the absence of glucose (Bueding and Potts, *vide* Bueding, 1949b) and that aqueous extracts of *Ascaris lumbricoides* esterify inorganic phosphate in the presence of fluoride (Rogers and Lazarus, 1949).

While the initial stages of the anaerobic carbohydrate utilization seem to be quite uniform, the terminal ones must show greater variability as indicated by the variety of endproducts. Little definite information concerning the exact mechanisms of their formation is available as yet. In *Litomosoides* an anaerobic dismutation of two moles of pyruvate to one mole of lactate, one mole of acetate, and one mole of carbon dioxide has been observed (Bueding, 1949a). The mechanism of fatty acid formation in worms like *Ascaris* has not yet been elucidated experimentally; a theoretical formulation has been presented by Jost (1928).

In the presence of oxygen the initial steps of the sugar degradation are the same as in its absence. Oxygen can enter the degradation chain at various points. The simplest case is that of the trypanosomes of the *evansi* group where (Marshall, 1948b) the only possible point is the oxidation of phosphoglyceraldehyde to phosphoglyceric acid, possibly through DPN and flavoenzymes. The same process is possible in any other oxygen-consuming and sugar-utilizing organism, but of greater importance in a variety of free-living organisms is the Krebs cycle.

Evidence for the presence of a Krebs cycle in parasites has been sought by offering the organisms intermediates of the cycle and studying whether or not these in-

intermediates significantly increase the oxygen consumption, or whether they speed up the decolorization of methylene blue in Thunberg experiments. The evidence bearing on this point is shown in table 6. It is obvious that malarial parasites and small nematodes have a well developed cycle although it may not in every respect be similar to that found in vertebrate material. Some observations possibly indicative of differences have been reported by Massey and Rogers (1949) in the case of small nematodes. Insofar as the large helminths are concerned, the currently available data are insufficient to allow a definite judgment as to the presence or the absence of such a cycle. No evidence for a Krebs cycle has been found in *Trichomonas* and *Trypanosoma lewisi*.

TABLE 5.—Anaerobic phase of glucose utilization in parasites

Emben-Meyerhof scheme of glucose breakdown		<i>Trypanosoma</i> <i>cruzi</i> (1)	<i>Trypanosoma</i> <i>lipticum</i> (2)	<i>Trypanosoma</i> <i>equiperdum</i> (3)	<i>Plasmodium</i> <i>gallinaceum</i> (4, 5)	<i>Plasmodium</i> <i>knowlesi</i> (6)	<i>Schistosoma</i> <i>mansoni</i> (7)
Glucose							
↓	← Hexokinase		x		x		
Glucose-6-phosphate		x	x		x		
↓	← Phosphohexoisomerase						
Fructose-6-phosphate		x			x		
↓	← Phosphohexokinase					x	
Fructose-1,6-diphosphate		x		x	x		
↙ ↘	← Aldolase		x	x	x		x
Phosphoglycer- aldehyde	← Dihydroxyacetone phosphate	x	x	x	x		
↓	← Triosephosphate- dehydrogenase		x	x	x		x
Phosphoglyceric acid		x	x		x		
↓	← Enolase						
Phosphopyruvic acid		x			x		
↓	← Phosphopyruvate- dephosphorylase	x				x	
Pyruvic acid					x	x	
↓	← Lactic dehydrogenase					x	x
Lactic acid					x	x	x

(1) Marshall, 1948b; (2) Harvey, 1949; (3) Chen and Geiling, 1946; (4) Speck and Evans, 1945; (5) Marshall, 1948a; (6) McKee, Ormsbee, Anfinson, Gelman, and Ball, 1946; (7) Bueding, 1949b.

CONCLUSION

The study of the carbohydrate metabolism of parasites began just about 50 years ago, the field being opened up by Weinland's classical papers (1901a, b). Considerable progress has been made in the intervening years as the present review has shown, but many problems remain to be solved. Some have already been indicated on the preceding pages, a few more may be mentioned as an outlook into the future.

It would appear likely that an elucidation of the types of carbohydrate utilization by various groups of parasites that have not yet been studied would be of importance. It is singular that the easily available trichomonads have so far received but little

TABLE 6.—Utilization of intermediates of the Krebs cycle by parasites

Species	Pyruvate	Oxaloacetate	Citrate	cis-Aconitate	α -Ketoglutarate	Succinate	Fumarate	Malate	Author
Protozoa									
<i>Trichomonas hepatica</i>	—					—			Willems, Massart, and Peters, 1942
<i>Trypanosoma evansi</i>	(x)					—	—		Marshall, 1948b
<i>Trypanosoma hippicum</i>	—	—	—			—	—	—	Harvey, 1949
<i>Trypanosoma lewisi</i>	(x)				(x)	(x)	(x)		Moulder, 1948a
<i>Plasmodium gallinaceum</i>	x	x	(x)	x	x	x	x	x	Speck, Moulder, and Evans, 1946
<i>Plasmodium lophurae</i>	x					x	x		Bovarnick, Lindsay, and Hellerman, 1946
Helminths									
<i>Fasciola hepatica</i>			—			x		x	Pennoit-DeCooman and van Grembergen, 1942
<i>Moniezia benedenti</i>			—			x		x	Pennoit-DeCooman and van Grembergen, 1942; van Grembergen, 1944
<i>Nematodirus</i> spp.	x	x		x	x	x	x	x	Massey and Rogers, 1949
<i>Ascaridia galli</i>	x	x		x	x	x	x	x	Massey and Rogers, 1949
<i>Neoplectana glaseri</i>	x	x		x	x	x	x	x	Massey and Rogers, 1949

x Compound utilized at significant rate.

(x) Compound utilized at insignificant rate.

— Compound not used.

attention, and that the acanthocephala that are so well adapted to parasitic life have been completely neglected, as have been such interesting parasites as the sacculinids and their allies. A study of the endproducts of carbohydrate utilization and the intermediate metabolism of these forms would round out the hitherto available data to a considerable degree.

Another important point which surely will come to the foreground in the future is the question of carbon dioxide fixation. Searle and Reiner (1940, 1941) observed a pronounced influence of CO₂ on the aerobic metabolism of *Trypanosoma lewisi* and an actual CO₂ fixation under anaerobic conditions. Moulder (1948a), however, while not denying a possible role of CO₂ could not find a similar influence on the aerobic metabolism in the same parasite. In worms, Rogers and Lazarus (1949) noted a decrease in bicarbonate content of the medium in excess of the CO₂ expelled by acid formation during anaerobic incubation of *Nematodirus* and *Ascaridia*.

Other interesting problems could be listed, and there is no doubt that the field of carbohydrate metabolism will yield important results in the future.

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THE OXYGEN REQUIREMENTS OF PARASITES

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The importance of oxygen in the life of higher animals was clearly demonstrated by Lavoisier only a few years after its discovery by Scheele and Priestley. Respiration was then defined as the utilization of oxygen and the production of carbon dioxide and water, and the presence of molecular oxygen was considered absolutely essential for life. It was not until many years later that Pasteur discovered that some bacteria can grow and thrive in the complete absence of oxygen. Since the time of Pasteur, it has been shown that many forms of life can also exist in environments containing no, or only a very little, oxygen, and our definitions of respiration have gradually broadened. Perhaps the most general definition is that respiration is the sum total of the chemical reactions carried out by the living cell which result in the liberation of energy (Stephenson, 1949).

Of these energy-yielding reactions, by far the most important are those in which foodstuffs are oxidized. If molecular oxygen is the ultimate oxidizing agent, the respiration is aerobic; if a substance other than oxygen is the final oxidizing agent, the respiration is anaerobic. In both aerobic and anaerobic respiration, the mechanism of oxidation is the same. Oxidation-reduction catalysts, the respiratory enzymes, mediate the transfer of hydrogen and electrons from the substance oxidized to the substance reduced (see Goddard, 1945; Green, 1940; Oppenheimer & Stern, 1939). The early and divergent ideas of Wieland and Warburg concerning the manner in which molecular oxygen reacts with substrates have long been reconciled in the now generally accepted view that the electrons and hydrogen of substrates are activated by dehydrogenases and that molecular oxygen, after being activated by cytochrome oxidase, accepts these electrons and hydrogen atoms and is reduced to water. In typical aerobic respiration, electrons and hydrogen are transferred from substrates to molecular oxygen through the intermediation of pyridinoprotein dehydrogenases, flavoproteins, cytochromes, and cytochrome oxidase. This complete system functions in the cells of higher animals, and the parasitic protozoa and helminths contain similar respiratory enzymes, but some parasites do not contain all of these catalysts and some possess still other oxidative enzymes of as yet unknown chemical nature. Typical anaerobic cells usually lack some of the respiratory enzymes, particularly the iron-porphyrin catalysts of the cytochrome-cytochrome oxidase system. In anaerobic respiration, the oxidation of one substrate is coupled with the reduction of another substrate through the intervention of the proper dehydrogenase systems.

The mechanism of aerobic respiration in malarial parasites is similar to that of their vertebrate hosts. The lactic (Speck and Evans, 1945) and malic (Speck, Moulder and Evans, 1946) dehydrogenases of *Plasmodium gallinaceum* require diphosphopyridine nucleotide for maximum activity, and flavin adenine dinucleotide is probably formed by *P. knowlesi* (Ball *et al.*, 1948), suggesting the presence of both

pyridinoproteins and flavoproteins in plasmodia. The oxygen uptake of several species of *Plasmodium* is almost completely inhibited by low concentrations of cyanide (Christophers & Fulton, 1938; Wendel, 1943; McKee *et al.*, 1946; Bovarnick, Lindsay & Hellerman, 1946), and the respiration of *P. knowlesi* is lessened in the presence of carbon monoxide (McKee *et al.*, 1946). These observations indicate that the malarial parasites utilize oxygen by means of a heavy metal respiratory enzyme. It has been tacitly assumed that this enzyme is the iron-porphyrin cytochrome oxidase, but actual proof of the point is still lacking, since enzymes other than iron-porphyrins may also be inhibited by cyanide and carbon monoxide.

Mammalian trypanosomes may be divided into two groups according to the effect of cyanide on their aerobic respiration (von Brand & Johnson, 1947). The oxygen uptake of the members of the non-pathogenic or moderately pathogenic *lewisi* subgroup, *T. lewisi*, *T. cruzi*, and *T. conorhini*, is almost completely suppressed by 0.001 M cyanide. In contrast, the oxygen uptake of the bloodstages of the pathogenic trypanosomes of the *evansi* and *brucei* subgroups, *T. equiperdum*, *T. hippicum*, *T. brucei*, *T. rhodesiense*, and *T. gambiense*, is unaffected or even stimulated by this concentration of cyanide. The line of division appears to be a very fundamental one, for it coincides with the two groups of trypanosomes established by Hoare and Coutelen (1933) on the basis of differences in life cycle and morphology. In addition, the cyanide-insensitive trypanosomes utilize glucose several times faster than the cyanide-sensitive trypanosomes (see von Brand & Johnson, 1947). Krijgsman (1936) has postulated that the trypanosomes of the *evansi* subgroup have evolved from their insect-inhabiting ancestors through the loss of all enzymes except those associated with hexose breakdown and protein synthesis. Many of these supposed trypanosome ancestors are known to be cyanide-sensitive (Lwoff, 1934) like the trypanosomes of the *lewisi* subgroup, and it makes an attractive hypothesis to assume that the pathogenic trypanosomes have, through adaptation to a thoroughly parasitic life, become cyanide-insensitive by the loss of a heavy metal respiratory enzyme. However, the cyanide-insensitive trypanosomes actually consume oxygen faster than those of the *lewisi* subgroup, despite their lack of a heavy metal catalyst. Thus, in trypanosomes, the shift to a cyanide-insensitive respiration must also have been accompanied by the appearance of a new respiratory enzyme capable of being oxidized as fast or even faster than the original heavy metal enzyme.

The presence of dehydrogenases in parasitic helminths is indicated by observations that *Ascaris lumbricoides* (Laser, 1944) and several species of flatworms (Pennoit-de-Cooman & Van Grembergen, 1942) carry out the enzymic reduction of methylene blue. Succinate greatly accelerates the rate of methylene blue reduction, indicating the presence of succinic dehydrogenase. At present, there is only indirect evidence for the presence of flavoproteins in parasitic helminths (see Bueding, 1949a); the flavin content of the few worms which have been analyzed is quite low (Gourevitch, 1937). Although, as first observed by Keilin, many helminths exhibit the absorption bands characteristic of the cytochromes when examined in the spectroscope (see Bueding, 1949a), it is not known whether or not the pigments responsible for these absorption bands actually function as respiratory catalysts. In *Litomosoides carinii*, the one helminth which has been directly assayed for cytochrome c activity, none could be detected (Bueding, 1949b). The sensitivity of helminth respiration to cyanide shows great variation, but the parasitic worms

may be roughly divided into cyanide-sensitive and cyanide-insensitive groups (Table 1). The cyanide-sensitive enzymes are undoubtedly heavy metal proteins, but not necessarily cytochrome oxidase. In fact, Bueding (1949b) has shown that although the respiration of *Litomosoides carinii* is strongly inhibited by both cyanide and carbon monoxide, extracts of this filarial worm fail to perform the specific enzymatic function of cytochrome oxidase, the reduction of cytochrome c. Thus, it is probable that *Litomosoides*, like the pathogenic trypanosomes, contains a highly active auto-oxidizable respiratory enzyme of an unknown chemical nature. Many parasitic nematodes contain iron-porphyrin pigments closely related to hemoglobin both in chemical structure and in the ability to combine reversibly with molecular oxygen (reviewed by Hobson, 1948). These hemoglobin-like pigments are undoubtedly synthesized by the worms themselves and not by their hosts. They differ from vertebrate hemoglobins in absorption spectra and oxygen saturation curves and the worm hemoglobin is still present in individuals maintained *in vitro* for many weeks. However, it is not known at present whether or not these pigments play a functional role in the distribution of oxygen within the tissues of parasitic nematodes.

TABLE 1.—Cyanide Sensitivity of Parasitic Helminths

Cyanide sensitive—Respiration strongly inhibited by 0.001 M HCN or less	
<i>Trichinella spiralis</i> (larvae)	Stannard, McCoy & Latchford, 1938
<i>Litomosoides carinii</i>	Bueding, 1949b
<i>Schistosoma mansoni</i>	Bueding, 1949a
Cyanide insensitive—Respiration only partially inhibited by 0.01–0.001 M HCN	
<i>Diphylobothrium latum</i>	Friedheim & Baer, 1933
<i>Taenia taeniocormis</i>	Willmoth, 1945
<i>Moniezia benediti</i>	Van Grembergen, 1944
<i>Ascaridia galli</i>	Rogers, 1948
<i>Nippostrongylus muris</i>	Rogers, 1948
<i>Ascaris lumbricoides</i>	Laser, 1944

The energy released in the biological oxidation of foodstuffs is not entirely dissipated as heat, but probably about half of the energy released by oxidations is conserved, stored, and transferred in the form of high-energy phosphate bonds (see Lipmann & Kaplan, 1949). In both aerobic and anaerobic oxidations, the energy so released may be used to convert inorganic phosphate into high phosphate-bond-energy compounds. This high energy phosphate may then be used to form adenosine triphosphate. In this reactive compound, phosphate-bond-energy is available for a variety of metabolic purposes. As will be discussed by Dr. von Brand today, phosphorylations and phosphorylated intermediates are of great importance in the economy of animal parasites.

Although the essential mechanisms of aerobic and anaerobic respiration are the same, the energy obtained from the oxidation of a substrate under anaerobic conditions is much less than in the presence of oxygen. For example, the useful energy liberated from the glycolysis of glucose to lactate (about 60 kg. cal. per mole glucose) is only about one-tenth the amount formed by the complete aerobic oxidation of glucose to carbon dioxide and water (about 600 kg. cal. per mole glucose). Even when aerobic oxidations are incomplete and do not proceed all the way to carbon dioxide and water, a very common occurrence in animal parasites, the yield of energy per mole of substrate is still greater in the presence of oxygen. This difference in energy yield is undoubtedly related to the fact, first observed by Pasteur, that organisms with active aerobic and anaerobic respirations utilize oxidizable carbohydrates and

accumulate partially-oxidized endproducts more slowly in the presence of oxygen than in its absence, thus obtaining the same amount of useful energy from the more complete oxidation of a smaller amount of substrate (for discussion of the Pasteur effect, see Lipmann, 1942). This Pasteur effect is well-illustrated in the earthworm, in which the anaerobic utilization of glycogen and formation of valeric acid is much greater than the aerobic rate, despite the fact that aerobic oxidation in the earthworm is not complete (Lesser, 1908, 1910). Therefore, as compared with aerobic metabolism, anaerobic metabolism is characterized by a rapid utilization of oxidizable substrates and a pronounced accumulation of partially-oxidized endproducts.

In their relationship to molecular oxygen, the different forms of life are often divided into three groups: the obligate aerobes, the obligate anaerobes, and the facultative anaerobes. Obligate aerobes require ready access to molecular oxygen and do not survive indefinitely in its absence. Oxygen is actively toxic to obligate anaerobes and they grow only in the complete absence of oxygen. Facultative anaerobes survive more or less indefinitely either in the presence or absence of oxygen. This is a simple and straightforward classification, but it is difficult to definitely assign many animal parasites to any one of these categories.

The blood forms of trypanosomes, however, appear to be real obligate aerobes. von Brand (1933) found that trypanosomes survive only a few hours under completely anaerobic conditions. It has never been determined whether or not malarial parasites can long survive complete anaerobiosis. Plasmodia are usually cultivated *in vitro* in the presence of oxygen tensions equal to that of alveolar air (Trager, 1941, 1943; Geiman *et al.*, 1946; Ball *et al.*, 1948), but *P. knowlesi* may be cultured equally well under much lower oxygen tensions (Anfinson *et al.*, 1946). Ball and his associates have also found that the *in vitro* multiplication of *P. knowlesi* is accompanied by a much smaller increase in oxygen uptake than is observed during a comparable period of growth *in vivo*, suggesting that the energy released in the anaerobic breakdown of glucose may be used to meet most of the energy requirements for the *in vitro* growth of *P. knowlesi*, but it still remains to be determined to what extent the normal rate of parasite growth *in vivo* is dependent upon aerobic oxidations. In general, parasitic worms have a remarkable resistance to oxygen lack, but when their oxygen requirements are more carefully investigated, it may be found that many of them, such as the small nematodes recently studied by Rogers (1949), require at least some oxygen for indefinite survival and growth. From his studies on the effect of cyanine dyes on *Litomosoides carinii*, Bueding (1949b) has recently concluded that aerobic respiration is essential for the survival of this helminth. It is a curious but well-established fact that the eggs of many nematodes require relatively high oxygen tensions for development into adult worms which may be more or less indifferent to the presence of oxygen (for references, see von Brand, 1946, p. 78). It has been suggested that obligate aerobes survive only in the presence of molecular oxygen because they lack the proper enzymic equipment for obtaining an adequate energy supply under anaerobic conditions, but trypanosomes, malarial parasites, and *Litomosoides*, all organisms proven or suspected of being obligate aerobes, rapidly break down glucose in the absence of oxygen. Other possible explanations for an absolute oxygen requirement may be that certain essential metabolites are formed only in reactions involving molecular oxygen, or that a toxic metabolite may accumulate during anoxymbiosis, but no definite examples of such occurrences have yet been reported.

Obligate anaerobes are unable to survive in the presence of molecular oxygen, and oxygen is rapidly toxic to them. The classical examples of obligate anaerobes are the bacteria of the genus *Clostridium*, and most of our general ideas concerning the nature of oxygen toxicity are based on studies with these bacteria. True obligate anaerobes are relatively rare among the animal parasites. After a very thorough survey of the oxygen requirements of invertebrates, von Brand (1946) concluded that among the parasitic forms only the rumen ciliates, Westphal, 1934; Hungate, 1942) and the flagellates inhabiting the intestines of termites (Cleveland, 1925; Trager, 1934) are rapidly killed by ordinary oxygen tensions. Work on the culture of *Endameba histolytica* suggests that it may also be an obligate anaerobe (see Chang, 1948), but recent measurements of oxidation-reduction potentials in such cultures indicate that *E. histolytica* can survive in the presence of very low oxygen tensions (Jacobs, personal communication). However, parasites which are not obligate anaerobes may be injured by high oxygen tensions approaching that of pure oxygen. For example, high oxygen tensions inhibit both the respiration (Silverman *et al.*, 1944; McKee *et al.*, 1946) and growth (Trager, 1941; Anfinsen *et al.*, 1946) of

TABLE 2.—Possible Mechanisms of Oxygen Toxicity

Mechanism	Example of this type of reaction which is known to occur in biological material
1. Oxygen may oxidize some essential enzyme, growth factor, or substrate which is active only in the reduced state.	1a. hemoglobin (Fe ⁺⁺) $\xrightarrow{O_2}$ methemoglobin (Fe ⁺⁺⁺) 1b. ascorbic acid $\xrightarrow{O_2}$ dehydroascorbic acid 1c. cysteine $\xrightarrow{O_2}$ cystine
2. Oxygen may form a loose, inactive complex with some essential enzyme, growth factor or substrate.	2. hemoglobin $\xrightarrow{O_2}$ oxyhemoglobin
3. Oxygen may participate in a reaction producing a toxic substance.	3. reduced flavoprotein $\xrightarrow{O_2}$ oxidized flavoprotein + H ₂ O ₂

malarial parasites, inhibit the respiration of trypanosomes (Moulder, 1948) and kill adult *Ascaris* (Laser, 1944). Although it is unlikely that the basis for oxygen toxicity is the same in all these organisms, it is convenient to discuss the deleterious effect of oxygen upon both aerobes and anaerobes at the same time. Theoretically, oxygen may attack an organism in several different ways. The accompanying table is based on the assumption that oxygen, like any other inhibitor, exerts its effect by reacting with a portion of the cell structure. The fact that the inhibitory effect of oxygen greatly increases with its concentration suggests that it is entering into a reaction obeying the mass law. Since oxygen is a very reactive substance, it is not surprising that it may react with some types of cells in such a way as to interfere with their normal function. Elucidation of the mechanism of oxygen toxicity, like elucidation of the mechanism of chemotherapeutic action, depends mainly upon knowledge of the enzymic composition of the affected organisms. Despite our meagre knowledge of the enzymic architecture of animal parasites, one example of oxygen toxicity has been successfully explained. Laser (1944) has shown that *Ascaris* is killed at high oxygen tensions by the accumulation of hydrogen peroxide at a faster rate than it can be destroyed by the very small amount of catalase present in this nematode (Lesser, 1906). However, it is unlikely that this is a general mechanism of oxygen toxicity. For example, malarial parasites are injured by

oxygen while living within erythrocytes, long the standard starting material for preparation of catalase.

A facultative anaerobe has already been defined as an organism capable of maintaining itself for a more or less indefinite period either in the presence or in the absence of molecular oxygen. By this definition, most parasitic protozoa and worms are facultative anaerobes, but it is not surprising that this large group of organisms is made up of several quite diverse metabolic types. Some of these are illustrated in Table 3 which compares the effect of oxygen on the metabolism of three representative helminths. *Eustrongylides* might be called a "true facultative anaerobe." Like the free-living earthworm, it has an active aerobic and anaerobic respiration and exhibits a pronounced Pasteur effect. It may reasonably be assumed that *Eustrongylides* is capable of deriving useful energy from either aerobic or anaerobic oxidations, depending upon the oxygen tension of its environment. *Ascaris* is capable of consuming oxygen (von Brand, 1934; Laser, 1944), but it only slightly reduces its rate of glycogen consumption and acid production when placed under aerobic conditions. It is not certain that *Ascaris* derives absolutely no benefit from

TABLE 3.—Effect of Oxygen on the Metabolism of Parasitic Helminths

Organism	Mg. per 100 gm. worms per day		
	Aerobic	Anaerobic	$\frac{\text{Anaerobic}}{\text{Aerobic}}$
<i>Eustrongylides</i> larvae ¹			
Glycogen used	300	900	3.0
Acid formed (as valeric acid)	0	300	—
<i>Ascaris lumbricoides</i> adults ²			
Glycogen used	1200	1400	1.2
Acid formed (as valeric acid)	160	220	1.4
<i>Schistosoma mansoni</i> adults ³			
Glucose used	3500	3500	1.0
Lactic acid formed	3100	3100	1.0

Some of the data have been recalculated in different units for purposes of comparison.

¹ von Brand, 1938.

² von Brand, 1934.

³ Bueding and Oliver-Gonzales, 1948.

its aerobic respiration, but obviously even in the presence of oxygen, *Ascaris* continues to gain most of its energy from the anaerobic oxidation of carbohydrate. Finally, *Schistosoma mansoni* is completely indifferent to oxygen. Although it consumes some oxygen, *Schistosoma* seems to obtain all of its energy from anaerobic respiration. However, since it is not poisoned by oxygen, *Schistosoma* can live under aerobic conditions and is classified as a facultative anaerobe.

In conclusion, we have seen that parasites differ widely in their response to molecular oxygen. For some, the presence of oxygen is absolutely essential for their survival. Others are injured or even killed by oxygen. Some parasites preferentially use oxygen as an oxidizing agent in their respiration but are able to survive without it. Still other parasites, while not injured by oxygen, fail to use aerobic oxidation as an effective energy source. Thus, the relationship of a specific parasite to molecular oxygen is determined, not only by the availability of oxygen in the natural environment of the parasite, but also by the enzymic constitution of the parasite itself. Since the days of Bunge, there has been much speculation on the effect of parasitic adaptation upon the enzymes of parasitic organisms (see von Brand, 1946, p. 279). Many have felt that parasitic adaptation has been accompanied by the loss of aerobic respiratory enzymes and a trend toward an anaerobic

mode of existence. In some cases this may be true, but there are aerobes among the animal parasites and anaerobes among related free-living types, and the sequence of biochemical evolution is not clear.

The exact answer to the question of parasitic adaptation and enzyme loss and to all questions concerning the oxygen requirements of parasites cannot be given now. Satisfactory answers will require, first, a detailed study of the chemical nature of the respiratory enzymes of a variety of parasitic and related free-living forms, and second, more extensive investigation of the energy requirements of parasites cultured *in vitro* under definitely known conditions of aero- and anaerobiosis. The wide variety of respiratory mechanisms possessed by the parasitic protozoa and helminths should make the results of such studies of general interest to all biologists.

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EFFECT OF DRUGS ON METABOLISM AND ENZYME SYSTEMS OF PARASITES*

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Knowledge about the action of drugs is as old as the history of mankind. Already primitive man observed the effect of certain herbs and plants on the course of disease. Undoubtedly, much of this information was obtained by selfmedication through a process of trial and error. Up to the present, such an empirical procedure has been used in the search of new chemotherapeutic agents although the synthetic activity of nature has been replaced by that of the organic chemist and animals have replaced human beings in such experiments. Yet, the basic philosophy of trial and error has continued essentially unchanged.

Until some time ago, discussion of chemotherapeutic agents against parasitic diseases was limited mainly to a consideration of apparent usefulness in human therapeutics. Formerly, concepts of mechanisms of action, even now but rarely elucidated in any detail, were in an even more primitive state. Only in rare cases was it possible to direct investigations of the action of drugs toward basic mechanisms occurring *within* the parasite because little was known about the fundamental chemical processes of cytoplasm. As these processes have begun to be clarified, information can be acquired concerning the manner in which drugs affect and alter metabolic systems of parasites. Such an approach has been advocated in 1946 by W. H. Wright (1) who stated that to date experimental work on the chemotherapy of parasitic diseases has been superficial in character and "that there is a definite need for a more fundamental type of research to augment methods now being employed in the development of new compounds. For instance, we should know more concerning the physiology of the parasites against which we aim our weapons so that we may strike at more vulnerable points."

The evidence available today, although in some cases circumstantial, suggests the possibility that the action of many chemotherapeutic agents is due to a direct or indirect effect on enzyme systems. This is by no means a new hypothesis. Already in 1914 Simon and Wood (2) suggested that the toxic action of certain dyes might be due to their interference with intracellular enzymes.

The chemotherapy of infectious and parasitic diseases is based on the use of compounds which, in suitable dosage, are more damaging to the invading organism than to the host. Translating this principle into biochemical language one may define a chemotherapeutic agent as a compound which inhibits essential metabolic reactions of the pathogenic organism to a greater degree than those of the host. Chemotherapeutic agents vary in their margins of safety from those that are of almost the same order of toxicity for both host and parasite to those that are essentially devoid of

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deleterious effects on any mammalian host. Examples of such extremes are actually to be found among drugs which may be used in the treatment of a single disease, like syphilis. Thus, metallic mercury, when used by inunction, is of such toxicity that it is possible only to arrest the progress of the infection, while with penicillin not only cures are produced but man appears to be essentially uninjured by any dose so far administered.

The fact that para-aminobenzoic acid abolishes the antibacterial action of sulfonamides indicates that interference with metabolic processes of the invading organism may result from the use of compounds structurally related to, but not utilizable in place of substances essential for life and reproduction. This type of interference can be explained most simply by a direct competition between the essential substance and its structural analogue for some cellular component for which they both have great affinity. Such essential substances, utilization of which is antagonized competitively by structural analogues, have been called "essential metabolites." As pointed out by Green (3), this term is rather misleading because it implies that such substances serve as substrates of enzymes. In the case of para-aminobenzoic acid, it merely indicates that it *participates* in essential metabolic reactions as a catalyst because it is only required in extremely low concentrations. The exact role of para-aminobenzoic acid in these metabolic reactions, is not known, but unquestionably these reactions are enzymatic in nature and sulfonamides interfere with them by competitive inhibition, a well known phenomenon in enzyme chemistry.

The mechanism of the trypanocidal action of trivalent arsenicals has been the subject of many investigations. Arsenicals affect functional rather than structural characteristics of the cell. The observations which led to such a concept were made in 1923 by Voegtlin, Dyer, and Leonard (4) who demonstrated that compounds possessing a free sulfhydryl group, such as glutathione or cysteine decreased markedly the trypanocidal activity of arsenicals *in vitro* and *in vivo*. These findings indicated that arsenoxides combine readily with sulfhydryl groups. The protection from the effects of arsenic by glutathione would then be due to the affinity of its sulfhydryl groups for arsenic. In this manner, the presence of glutathione or of cysteine would result in a combination with the arsenical and would prevent it from combining with functionally important proteins of the parasite. The affinity of monothiols for arsenicals brought up the question whether or not arsenicals combine also with sulfhydryl groups of cell proteins. During the past 10 years it has been demonstrated that intact sulfhydryl groups are essential for the proper functioning of many enzymes (5, 6, 7, 8, 9). When such enzymes are inhibited by low concentrations of trivalent arsenicals, iodoacetate or p-chloromercuric benzoate, free sulfhydryl groups are no longer detectable. Therefore, whenever blocking of sulfhydryl groups occurs, these enzymes are inactivated. Furthermore, a close correlation exists between enzymes requiring intact sulfhydryl groups for their proper functioning and the action of arsenicals on enzymes (8, 9). Those enzymes which are inactivated when their sulfhydryl groups are blocked are readily inhibited by low concentrations of an arsenical. On the other hand, the activity of those enzymes which lack or do not require intact sulfhydryl groups is not affected by arsenicals. Therefore, the chemotherapeutic effect of trivalent organic arsenicals is due to their selective effect on enzymes for which intact sulfhydryl groups are essential for their functional integrity.

Recent observations of Chen (10) and of Marshall (11) have supplied indications about the particular enzyme system of trypanosomes which is affected by arsenicals. The first step in the utilization of carbohydrate consists in the phosphorylation of glucose by adenosinetriphosphate. This reaction is catalyzed by hexokinase, a sulfhydryl enzyme. The activity of hexokinase of *T. evansi* and of *T. equiperdum* is strongly inhibited by trivalent arsenicals. Since a high rate of glucose utilization is an essential requirement for these organisms, inhibition of their hexokinase could explain the trypanocidal effects of arsenicals.

Not all species of trypanosomes are sensitive to arsenicals. For instance, they have no effect on *T. cruzi* *in vivo*. This agrees with the observations of Von Brand (12, 13) that the respiration of this parasite is inhibited by arsenicals to only a slight degree. Unless *T. cruzi* is impermeable to arsenicals it is probable that its essential metabolic reactions are catalyzed by enzymes whose functional integrity is not dependent on intact sulfhydryl groups.

The above discussion of the metabolic effects of arsenicals has been limited to compounds containing trivalent arsenic. With regards to the chemotherapeutic activity of pentavalent arsenicals there is general agreement that their effect *in vivo* is due to their conversion by the host to trivalent arsenicals (4, 1, 14).

No evidence is available that arsenic-resistant strains of trypanosomes have developed enzyme systems or metabolic pathways which are not inhibited by arsenicals and which are not dependent on sulfhydryl enzymes (15). Nor is it possible to explain arsenic resistance by an increase, within the parasite, of the number of sulfhydryl groups which might bind arsenic and thereby protect essential sulfhydryl groups of enzymes (16). On the other hand it has been shown conclusively that the permeability of arsenic resistant strains for arsenicals is greatly reduced (17, 18, 19, 20, 21).

Indications are available that the mechanism of the chemotherapeutic action of antimonials is similar to that of arsenicals. Cysteine reduces the trypanocidal activity of antimonials (22) as well as their inhibitory effect on the rate of utilization of glucose by *T. equiperdum* (23). Furthermore, British anti-lewisite (BAL) prevents and reverses the inhibitory effects of 'Fuadin' on the respiration and on glycolysis of *Schistosoma mansoni* (24).

A number of observations concerning the metabolic effects of non-metallic trypanocidal agents have been reported. Marshall (11) has found that low concentrations of the aromatic diamidine stilbamidine inhibit the utilization of pyruvic acid by *T. evansi*. If pyruvate is converted to amino acids by these trypanosomes a reduction in the metabolism of pyruvic acid would result eventually in an inhibition of protein synthesis and ultimately in a suppression of growth. This would be in agreement with the findings of Lourie and Yorke (25) that aromatic diamidines are trypanocidal *in vitro* only after a period of incubation for 24 hours. It appears that stilbamidine in low concentration produces denaturation of nucleoproteins and a dissociation of nucleic acids from proteins (26, 27). Whether this effect is related to the trypanocidal action of aromatic diamidines has not been investigated.

Town, Wills and Wormall (28) have reported recently that suramine (Bayer 205) in very low concentration inhibits the fermentation of glucose by yeast. These authors imply that the trypanocidal action of Bayer 205 similarly might be due to its effect on the anaerobic metabolism of glucose by trypanosomes. However, this

hypothesis is not supported by earlier work indicating that Bayer 205 had no effect on the metabolism of *T. brucei* even after prolonged incubation (29, 30). On the other hand 6 hours after injection of Bayer 205 in mice infected with *T. brucei* the respiration of the trypanosomes is reduced while their rate of anaerobic glucose utilization is not affected (29, 30). This would indicate that in the mammalian host Bayer 205 is metabolized to a compound possessing direct trypanocidal activity while the drug itself has no such effect *in vitro*.

By contrast, as shown by Ball (31) and Geiman (32), quinine, atabrine and chloroquine suppress the growth of *P. knowlesi* *in vitro* in similar concentrations as *in vivo*. Thus these drugs themselves rather than their degradation products in the body exert antimalarial action. On the other hand, recent observations of Geiman (32, 33) indicate that degradation products of paludrine *in vivo* rather than paludrine itself exert antimalarial activity. Quinine produces a moderate inhibition of respiration of *P. gallinaceum* in concentrations equal to or only slightly above those observed *in vivo* after the administration of chemotherapeutically effective doses (34, 35). Furthermore, Moulder (36) has observed that after the administration of subcurative doses of quinine to chickens infected with *P. gallinaceum* the rate of glucose oxidation by the parasites is reduced and the anaerobic utilization of this sugar is increased. Therefore, it appears that the mechanism of action of this drug is due to a depression of the aerobic carbohydrate metabolism of the parasite which results in a reduction of the amount of energy available for reproduction and survival.

Atabrine inhibits a number of enzymes whose prosthetic groups (or coenzymes) are flavins (37, 38, 39). This inhibition does not occur in the presence of an excess of flavin (38, 39). Because of the structural similarity of flavins with atabrine, it has been postulated that the latter exerts its antimalarial effect by inhibiting competitively flavin enzymes (38). However, inhibition of flavin enzymes is as marked or even greater with quinine and certain aromatic nitrogenous compounds having no antimalarial activity (37). Also, all these compounds, including atabrine, inhibit other enzymes which have no flavin as prosthetic groups (39). Furthermore, the concentration of atabrine necessary to inhibit flavin enzymes is considerably higher than that needed to produce an antimalarial effect *in vivo*. Thus, inhibition of flavin enzymes by atabrine does not explain the chemotherapeutic action of this antimalarial. As suggested by Ball, Geiman and co-workers (40) the antimalarial action of atabrine might be due to an interference with the synthesis of flavin essential to the parasite, rather than with the activity of flavin enzymes.

Wendel (41) has shown that the relative effectiveness of 2 hydroxy-1,4-naphthoquinones *in vivo* against *P. lophurae* parallels their ability to inhibit respiration of the parasites *in vitro*. As shown by Ball and co-workers (42) these naphthoquinones are powerful inhibitors of succinic dehydrogenase. This inhibition is produced by an interference with the interaction of cytochrome b with cytochrome c. Fieser and Heyman (43) have obtained further evidence that the chemotherapeutic activity of hydroxynaphthoquinones against *P. lophurae* *in vivo* is due to a depression of the oxidative metabolism of the parasites. Of 82 naphthoquinones studied, every compound which had high activity *in vivo* was a potent inhibitor of the respiration of the malaria parasites *in vitro*. Hydroxynaphthoquinones have high antimalarial activity in ducks, moderate activity in chickens and practically no activity in man. This is explained by the fact that serum albumin interacts with and inactivates these

compounds. The affinity of serum albumin for hydroxynaphthoquinones is greatest in man and lowest in ducks (44).

As yet no investigations have been carried out to determine whether any anthelmintic drug used clinically exerts its effect by inhibiting essential metabolic reactions of the parasite. Recent experimental evidence indicates, however, that the marked chemotherapeutic effectiveness *in vivo* of certain cyanine dyes against the filarial worm *Litomosoides carinii* (45, 46, 47, 48) is based on their inhibitory effect on the oxidative metabolism of this organism (45, 47, 48). In concentrations as low as $1.3 \times 10^{-7}M$ (1:20 million) certain cyanines inhibit the oxygen uptake of the filariae to an extent of about 35% and in four times greater concentrations their respiration is almost completely inhibited (49). This reduction of oxidative metabolism is associated with a compensatory increase in the rate of glycolysis (49). This effect of the cyanines is not limited to conditions *in vitro*. When cotton rats infected with *Litomosoides carinii* are treated with succurative doses of cyanine dyes the worms remain motile but their oxygen uptake is reduced and their rate of aerobic glycolysis is increased. (45, 49).

The cyanines are equally effective in reducing the oxygen uptake of *Schistosoma mansoni* *in vitro* and *in vivo* (50, 51). However, they have no chemotherapeutic activity whatsoever against this organism (50, 51). *Schistosoma mansoni* belongs to a group of helminths which have an extremely low requirement for respiratory metabolism (52). This explains why an almost complete inhibition of their oxygen uptake produced by the cyanines does not affect their survival or their reproductive functions. Similarly, the cyanines have no chemotherapeutic activity against those intestinal helminths whose oxygen requirements are low, while they are active against *Ancylostoma caninum* (53) and against *Necator americanus* (54) whose survival probably is dependent on a high rate of respiratory metabolism.

Recently evidence has been obtained that, in contrast to respiration, glycolysis supplies a major portion of the energy required by *Schistosoma mansoni* (50, 51). Certain naphthoquinones, for example, 2-methyl-1,4-naphthoquinone, inhibit the rate of glycolysis of these trematodes while they have little effect on their respiration (50, 51, 55).

These compounds have slight but definite chemotherapeutic activity against *S. mansoni* in mice, because they potentiate the effect of very small and otherwise ineffective doses of 'Fuadin' (50, 55). The rate of glycolysis of schistosomes removed from mice treated with this combination is markedly reduced (55). These observations indicate that the chemotherapeutic activity of such naphthoquinones is based on their ability to decrease the rate of glycolysis of *Schistosoma mansoni*. Because of partial inactivation by serum albumin, naphthoquinones possess low chemotherapeutic activity *in vivo* (55). However, this example demonstrates the principle that anthelmintic agents can be developed by studying the nature and the inhibitors of essential metabolic reactions of the parasite. 'Fuadin,' a much more effective chemotherapeutic agent in schistosomiasis when large doses are employed, inhibits glycolysis of schistosomes to a lesser degree than oxygen uptake (50, 51). Thus, this compound is very effective in reducing *aerobic* metabolism, which at best plays a minor role in the survival of the worms; inhibition of essential glycolytic processes, on the other hand, occurs only with concentrations of the drug approaching those capable of producing injury to the host. These findings parallel the well-known and

regrettable fact that clinically effective doses of 'Fuadin' frequently produce toxic reactions in the human host.

Baldwin (56) has shown that the anthelmintic effect of hexylresorcinol is due to its paralyzing action on the muscle of *Ascaris*. The contractile substance of mammalian muscle is actomyosin, a birefringent protein. We have observed recently that the addition of hexylresorcinol to isolated muscle fibers of *Ascaris* resulted in a reduction of their birefringence (57). Possibly the anthelmintic action of hexylresorcinol might be related to an effect on the contractile substance of the *Ascaris* muscle.

Biochemical characteristics of parasitic worms vary greatly from one species to another (52). In view of these differences, which are much more pronounced than those existing among vertebrates, it is not surprising that many drugs which are highly effective against one particular type of parasite, are completely inactive against others (1). Even among parasitic worms possessing many morphological similarities, profound metabolic differences may prevail. This is well illustrated by the marked chemotherapeutic activity of cyanine dyes against the filarial worm, *Litomosoides carinii* on the one hand (45, 46, 47, 48) and their complete lack of activity against two other adult filarial species, *Dirofilaria immitis* (58) and *Wuchereria bancrofti* (59). It was not possible to study the latter parasite, which resides in the lymphatic system of its human host, *in vitro*; hence, the possibility exists that the cyanine dyes were ineffective because of their failure to enter the lymph following their intravenous injection into man. In dogs, however, comparable doses of cyanine dyes administered by the same route bestowed on the lymph the ability to inhibit the respiration of *L. carinii* *in vivo* (50). *Dirofilaria immitis*, of course, was subjected to very high concentrations of cyanine dyes *in vivo*, since the drug was injected into the vascular system, the actual site of the infection; yet the parasite survived. The metabolic differences between *L. carinii* and *W. bancrofti* are reflected also in the fact that 'Hetrazan' possesses marked chemotherapeutic activity against the latter parasite *in vivo* (61) while its effect on the former is of a very low order, both *in vivo* and *in vitro* (62). In the case of yet another tissue nematode, *Dracunculus insignis*, low concentrations of the cyanines produce a considerable reduction of the oxygen uptake, but no compensatory increase in the rate of glycolysis (63). This suggests that *Dracunculus insignis* also is much less dependent on oxidative metabolism than is *Litomosoides carinii*.

It is generally assumed that the main energy producing reactions are similar in most animals and microorganisms. If this were the case, there would be few opportunities to inhibit catabolic reactions of the parasite without damaging the host. However, marked differences in the carbohydrate metabolism between the mammalian host and a variety of parasitic organisms have been observed. For example, only a few parasitic helminths produce lactic acid from carbohydrate. Most of them convert glucose into lower or higher fatty acids (52). Furthermore, the products of the anaerobic carbohydrate metabolism of certain trypanosomes are succinic, pyruvic, formic and acetic acid, ethyl alcohol and glycerol (64, 65, 66), but not lactic acid.

While the intermediary anaerobic and aerobic metabolism of carbohydrate of malaria parasites resembles closely that of mammalian tissues (67, 68), this appears to represent an exception rather than a rule among parasitic species. Even if the

products of catabolic reactions were the same, the enzymes which catalyse them might be different in the host and in the parasite and thus they might be sensitive to different types of inhibitors. This is illustrated by the observations of von Brand (12, 13, 69, 70) that respiration of *Trypanosoma cruzi* is affected only slightly by arsenicals and other sulfhydryl inhibitors while the oxygen uptake of other trypanosomes, such as *Trypanosoma rhodesiense* or *gambiense* is completely insensitive to high concentrations of cyanide but sensitive to arsenicals. Also in filarial worms which have a high rate of respiration, no cytochrome c or cytochrome oxidase activity can be detected (71).

Much greater differences between host and parasites may be expected to exist in the enzymatic reactions involving anabolic or synthetic reactions about which almost no information is available as yet. Since such reactions proceed at relatively slow rates, the organisms must be cultured aseptically outside the host for prolonged periods of time. In the past 15 years considerable progress has been made in establishing such conditions with many parasitic organisms although this has not been possible as yet with media of known chemical composition. Once this latter goal has been achieved, a study of the exact nutritional requirements of parasites will be feasible. Under such conditions, experiments can be designed, in which attempts are made to block the utilization of essential metabolites by competitive and non-competitive inhibitors. It is quite possible that the essential requirements of a given parasite for several factors may differ qualitatively and quantitatively from those of the host. Thus, under proper conditions of dosage, prevention or reduction of the utilization of such factors might injure the parasite, but not its host.

It should be pointed out that not every inhibitor of essential metabolic reactions in pathogenic organisms will be an effective chemotherapeutic agent, even if the toxicity of such compounds is low. Many of them may prove inactive *in vivo* because of interaction with, metabolic modification by or unfavorable distribution in the tissues of the host or because of slow absorption or rapid excretion of such substances. In some cases, these factors may be overcome by altering the chemical structure of the inhibitor in such a manner that no decrease or loss of intrinsic activity occurs and no increase of its toxicity develops. Since this will not always be possible, many results of a practical nature may not be predicted for the immediate future. Nevertheless, an opportunity is afforded to supplement and eventually to replace the presently prevailing empirical methods with a more rational approach to chemotherapy.

It may be concluded from this discussion that, as our understanding of enzymatic mechanisms is advanced, the fundamental actions of more and more known drugs will be elucidated and possibilities will arise for the logical development of effective chemotherapeutic agents.

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Protein Metabolism of Parasites*

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INTRODUCTION

Parasitologists, by necessity are world conscious. Several years ago this Society heard a brilliant address entitled "This Wormy World" (Stoll, 1947). If we might be permitted to paraphrase subsequent titles, this Society has heard in succeeding years about the systematic world (Van Cleave, 1948) and the parasitologists world (Faust, 1949). Today and in the form of a symposium, "the molecular world of parasites" is being reviewed.

The statement quoted frequently in elementary texts that parasites have degenerated in form and function and hence must live on or in another animal to survive, generally implies that parasites are less complex in their mode of life than their hosts. When one tries to duplicate *in vitro* the simple life of a parasite, the concept of a less complex mode of life for parasites is in urgent need of revision. The papers already given are ample evidence that the parasitic mode of life is highly specialized and intricate. A review in this paper of the "Protein Metabolism of Parasites" amplifies the complexity of parasite metabolism, and the way protein utilization is integrated with the major known phases of metabolic processes as they function simultaneously.

Proteins, those "primary or preeminent" plant and animal substances of high molecular weight are synthesized during growth and in general, constitute the structural matrix and functional enzyme systems of living matter. Collectively the simple and conjugated proteins make up plant and animal materials taken in or supplied as food by free-living and parasitic cells for the synthesis of new protein.

Without exception, analyses of animals reveal the presence of protein in every organ or tissue. Furthermore, proteins are the basis of important essential body functions, the production of energy and synthesis of cytoplasm for growth. The functions of certain proteins range from the transport of oxygen to tissues, the motive mechanism of muscle, to regulators of activity and carriers of immune defenses of the host (Tracey, 1948; Everett, 1946).

The remarkable specificity of proteins is best stated in the words of Northrop (1949). "Each tissue of each species of plant or animal can form its own special proteins, and these proteins are characteristic of the organ as well as of the species. It is probable, therefore, that millions of different proteins exist." The apparent infinite variety of proteins is no doubt the basis for specific differences between living things and for the occurrence of highly integrated systems of enzymes and genes

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peculiar to the species. Each cell synthesizes and perpetuates its own kind of protein by combined growth and genetic processes that are still to be fully explained.

The protein metabolism of the cell must be considered as only one of the "flow processes" of cellular metabolism (Lipmann, 1946) resulting from the utilization of amino acids and action of enzymes. Coupled reactions with carbohydrate and lipid oxidative metabolism supply the energy needed for the synthesis and degradation of protein (Figure 1). The nature of these coupled reactions is unknown, although phosphorylative reactions are probably involved. Conversely, the products of denatured or split protein are linked in a reaction chain with the Krebs' cycle or take part in a coupled reaction with carbohydrates and lipids by oxidative or transaminase reactions.

SCHEMATIC DIAGRAM OF CELLULAR METABOLISM

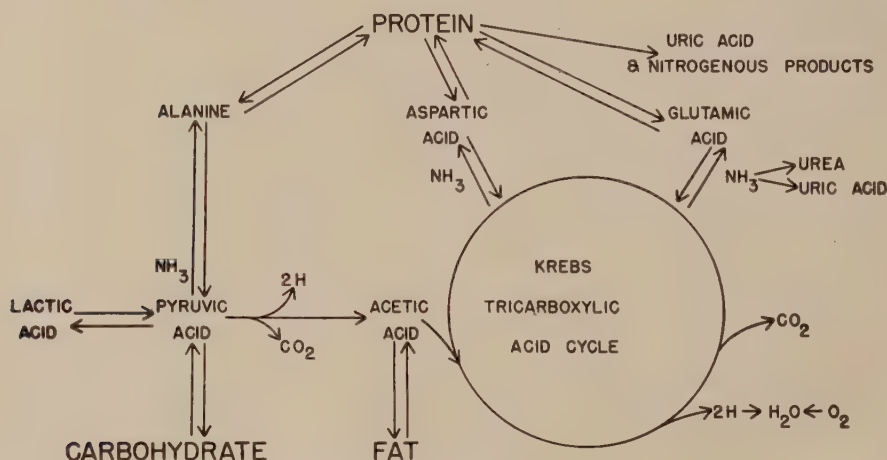


FIG. 1. Schematic Diagram of Cellular Metabolism.

This diagram represents the coupled reactions between carbohydrate, lipid and protein metabolism that make up cellular metabolism. The parasite can affect a host cell either by competing for available essential nutrients or by producing toxic waste products.

The excretory nitrogenous products of protein metabolism vary with the type of animal but the products are mainly ammonia, urea, uric acid, amino acids and other undetermined substances. Baldwin (1949) attached both environmental and evolutionary significance to the predominance of urea or uric acid as end products of nitrogen metabolism. These and other end products are also of pathological importance because of their toxicity. Some of the metabolic products of bacteria, for example, are excreted into the medium as proteases (Stephenson, 1949). The presence of intracellular proteases is of considerable importance to parasites because if they are absent, proteins of high molecular weight cannot be utilized and the required amino acids or peptides of low molecular weight must be made available from the environment for utilization by the cell for protein synthesis and growth. Herein lies one of the fundamental keys to the nature of the parasitic cell versus the host cell. Although much has been written about toxins produced by animal para-

sites, little evidence is available about the identity of nitrogeous excretory products and the degree and mode of action of their toxicity.

Time does not permit a detailed consideration of the mechanisms and importance of enzymes and amino acid breakdown to protein metabolism and growth of cells (reviewed by Barron, 1949). It is necessary to point out however, that the assimilative processes of the cell for amino acids are of importance not only for growth but they appear to be linked through the synthesis of protein and association of nucleic acids to the formation of nucleoprotein and the basic hereditary mechanism of the cell. These nucleoproteins are extremely important in cellular metabolism (Caspersson, 1947), in the gain or loss of virulence by pathogens (Avery et al, 1944) and in studies on the development of malignancy by normal cells (Stowell, 1946; Schneider, 1947).

Few comprehensive studies of protein metabolism in invertebrates have been carried out (von Brand, 1946). The investigations have been widely scattered in years and in the choice of organisms selected for study. Rather than attempt coverage of unrelated studies, some central theme was sought that would provide a basis for this review. In searching for a suitable theme, the diversity of parasites, their modes of life, types of host, parts of the host parasitized, degree of pathogenicity, and nature of and duration of immune response posed many pitfalls in arriving at an arbitrary choice of studies having something in common. Since blood and its elements play a considerable role in the nutrition, transmission and chemotherapy of parasites, studies of certain blood sucking arthropods, blood-dwelling or blood-sucking helminths and blood- or tissue-dwelling protozoa have been chosen for a comparative discussion. For that reason, then, this paper is not a comprehensive review but rather a discussion of key studies that appear to point the way for future experimentation and interpretation on the nature of parasitism.

Before proceeding, the following statement by Lipmann (1946) is to be emphasized and kept in mind. "Instead of the material being manipulated successively in spatially separated compartments, cellular chemistry involves a harmonious series of consecutive reaction steps which are brought about on a molecular scale by a host of catalysts, all present in the same reaction fluid." Herein lies the difficulty of isolating particular steps of cellular metabolism for study, so that experimentation will produce results enabling the interpretation of protein metabolism and its functional role in cellular processes.

Blood Sucking Arthropods

The evolutionary development of arthropods that feed on blood has brought these parasites in contact with a source not only of required nutrients but with a reservoir of infectious agents such as viruses, rickettsiae, bartonella, bacteria, spirochetes, protozoans and helminths. The ability of these infectious agents to adapt themselves and survive in arthropods, to undergo growth and cyclical development and to find their way back again into new hosts at the time the arthropod obtains a blood meal, involves a series of complex physiological processes. An understanding of the metabolism of the arthropod will make possible the correlation and interpretation of host-parasite physiology and contribute to our knowledge about the nature of parasitism.

Blood-sucking arthropods are particularly useful in metabolic studies because

of available biochemical tools for the identification of the hemoglobin and its products after digestion. The following figures give some idea of the quantities of blood available in certain species for study. The blood ingested by different species of mosquitoes varies from 0.7 to 3.9 mg. with female *Aedes aegypti* taking 1.5 to 1.7 times their original weight (Woke, 1937a; Hovanitz, 1947; Bates, 1949). In *Stomoxys*, sp., Lotmar (1949) showed that this fly ingested 13 mg. of blood or $1\frac{1}{2}$ times its body weight. The large reduviid bug, *Rhodnius prolixus*, will ingest blood up to 2 times its own weight of 80 mg., according to Wigglesworth (1943). This species because of its capacity for blood was chosen by Wigglesworth to study the fate of hemoglobin.

The findings of Wigglesworth on the fate of the hemoglobin in the blood meal are briefly summarized. Unfortunately no studies are available about the fate of the plasma. The hemoglobin becomes oxyhemoglobin at the start of the intestine, and a certain amount of the hemoglobin is absorbed unchanged into the hemolymph, an important observation for the possibility of large virus molecules being able to pass the gut membrane. In the gut, the molecule of hemoglobin is split into its components of heme and globin. The globin is absorbed, and acid hematin (ferric iron prophyrin) can be demonstrated unchanged as it is excreted along with uric acid. The pigment circulating in the body cavity changes greatly after the blood meal with heavy concentrations appearing in the salivary glands along with hemalbumin. In the pericardial cells, biliverdin possibly in the form of a chromoprotein was demonstrated. Another interesting point was the orange-red pigment to be found in eggs. The breakdown of the hemoglobin occurs largely in the epithelium of the gut and in pericardial cells. Heavy deposits of free iron could be detected in the gut wall and epithelium where it appears to accumulate throughout the life of the insect. In fact, this accumulation and its site appears to be analogous to that found in man and other mammals (Granick, 1946; Hahn, 1948). No free iron, biliverbin or urobilin could be demonstrated in the feces.

Studies of a similar type were done also by Wigglesworth (1948) with other blood-sucking arthropods. In the body louse, *Pediculus humanus corporis*, digestion takes place in the stomach, but a large fraction of the blood meal is undigested. Biliverdin was the only pigment detected in the pericardial cells. In the rat flea, *Ceratophyllus fasciatus*, the digestion of blood in the stomach is incomplete because the feces contain much unchanged hemoglobin. No bile pigment was detected in the gut, and the epithelial cells of the stomach and the malpighian bodies were colorless. In *Anopheles maculipennis* and *Aedes aegypti*, the digestion of blood in the gut is complete as evidenced by only small amounts of protohematin in the feces. In the tick, *Ornithodoros moubata*, protohematin was detected in the gut wall and alkaline hematin in both the hemolymph and eggs.

The comparative studies made by Wigglesworth (1929, 1931) on the digestive enzymes of three genera of insects are pertinent to this discussion. The results with *Chrysops silacea* (the intermediate host of *Loa loa*), *Glossina submorsitans* and *G. tachinoides* (vectors of African sleeping sickness) and *Calliphora erythrocephala* (a non-blood sucking fly) show an interesting predominance of carbohydrate splitting enzymes (amylase, invertase and maltase) in *Calliphora*, and the predominance of the proteolytic enzymes (tryptase and peptidase) in the species of *Glossina*. *Chrysops silacea* takes an intermediate position, having both types of enzymes pres-

ent. A pH of 6.4 and the presence of active proteolytic enzymes in the mid gut of *Glossina* where the cyclical development of the polymorphic trypanosomes takes place (Wenyon, 1926) suggests a biochemical or nutritional explanation for the development of these trypanosomes in *Glossina*. The split products of protein digestion would be available in highest concentrations at this site.

Experiments by Woke (1937 a and b) on the effects of blood fractions and various species of blood on egg production by adult *Aedes aegypti* are again indicative of the role of blood nutrients in a vital physiological process. Viable eggs were produced after the ingestion of whole blood, defibrinated, decalcified, or heparinized blood and different components of whole blood. The different results obtained after feeding the anophelines on erythrocytes of rabbit and chicken blood, point to possible quantitative differences in essential nutrients to be found in non-nucleated and nucleated red cells. The differences between plasma and serum of rabbits might be explained by the presence of larger quantities of hemoglobin in the serum because of the rupture of red cells during defibrination. The results when *A. aegypti* was fed on different species of blood are not clear cut. Similar experiments should be done using species of mosquitoes that are more host-specific in their feeding habits.

Numerous other workers have made similar studies (reviewed by Trager, 1947) with different species and stages of mosquitoes, and concluded that key nutrients and blood are needed for larval development and production of viable eggs by certain species. Even *Stomoxys calcitrans* requires blood for egg-production. In any case, more precise experiments could be done making use of artificial methods to obtain feeding of mosquitoes (Bishop and Gilchrist, 1946; Whitman, 1948) with synthetic plasma, plasma and red cell fractions.

Very few studies have been made on the excretory products resulting from the digestion of proteins in parasitic insects. One of the best series of experiments is that of Brown (1938) who studied the nitrogenous metabolism of the flesh-fly, *Lucilia sericata* (reviewed by Craig and Hoskins, 1940). Although no comparable dry-weight-data were given to enable a comparison of excretory products at the different developmental stages, the figures, nevertheless, are of great interest because they show the different amounts of excretory products that one might get from different stages in the life cycle. The larvae were grown on a sterile casein-yeast-salt mixture and lanolin diet and the insects and the fecal material were analyzed throughout the life cycle. At one stage or another, the common excretory products of protein metabolism could be detected. Nevertheless, uric acid is the predominant excretory product, and except for very young adults and the pupae, ammonia is not excreted *per se*. Urea was detectable only in excreta from the meconium, the one day adult and in analyses of the whole insect during larval stages. Allantoin is excreted in considerable quantities by larvae and young adults. These results identifying allantoin as a metabolic product recall the studies some years ago on the use of larvae of certain insects to promote the healing of wounds because of their excretion of this growth-promoting substance (Robinson, 1935).

A discussion of protein synthesis by arthropods would not be complete without reference to the studies of Zametchnik and his collaborators (1949) on the biological synthesis of radioactive silk. In realizing that silk is easily isolated as a stable protein and that it contains a high quantity of glycine and alanine, these workers conducted some experiments with silk worms, making use of C^{14} -labeled glycine

and alanine as an approach to the study on the synthesis of this very important protein. As a result of carefully controlled *in vitro* and *in vivo* studies with the isolated silk glands of the insect, Zamecnik *et al* were able to show that protein synthesis incorporating the labeled-amino acids had taken place under both experimental conditions. The exact nature of the protein synthesis has yet to be explained but this type of study is of far reaching importance to similar studies with radioactive isotopes. Similar studies could be done with a variety of blood-sucking and other arthropods of a parasitic nature, because the needed radioactive compounds that go into protein synthesis are becoming available in increasing numbers and quantities.

Helminths

In passing now to helminthic species and in keeping with the general theme, a consideration will be made of those helminths that feed on or live in blood. In seeking material about helminths for this review, few studies on the protein metabolism of helminths were found; a result noted also by Bueding (1949). When one considers the gross size of blood-sucking and blood-dwelling helminths and the quantity of blood that is apparently involved, the failure to investigate more fully the protein metabolism of helminths is indeed surprising.

Although much was written about the cause of anaemia in hookworm disease, it remained for Wells (1931) and Nishi (1933) to observe independently the sucking of blood by *Ancylostoma caninum*. These workers observed that a single worm would remove 0.7–0.8 cc. of blood from its host in 24 hours and Foster and Landsberg (1934) presented additional evidence to show that the anaemia of hookworm disease is purely of a hemorrhagic nature. In any case, the amount of blood consumed by hookworms is sufficient for microchemical studies of digestion and metabolism.

Rogers (1940) adapted microspectroscopic techniques to the direct examination of nematodes in an effort to determine whether or not blood is digested by these parasites and the nature of the changes undergone by hemoglobin. Direct examination of the gut contents of *Strongylus* sp. with a microscope gave little evidence of their nature. A technique was then developed to remove intact the intestines of female *Strongylus edentatus* for study. Forty specimens of this worm were examined and in each case a hemoglobin product was present. Calculations of the average amount of hemoglobin per worm to the original weight of the parasite gave figures that ranged from 0.73 to 1.56 per cent hemoglobin.

Three lots of *Strongylus vulgaris* were investigated. In every case, the examination revealed the bands of globin hemochromagen indicating that hemoglobin was present in the gut of the worm. When referred to the average weight of worms, the percentage of hemoglobin ranged from 0.18 to 0.80. The examination of the fourth stage larvae of *Strongylus vulgaris* also revealed that this stage was capable of digesting blood. This result suggested that blood may form a large proportion of the diet of these worms. Approximately 20 specimens of *Ascaris lumbricoides* from the pig were examined by a similar technique. Of these organisms only two were found to have hemoglobin products in their intestine. The failure to find blood in more of these parasites, suggested to Rogers that blood ingestion by *A. lumbricoides* was accidental. Examination of a few specimens of *Parascaris equorum*, failed to reveal any hemoglobin products in the intestines. The opposite however, was true

when an examination was made of the intestines of *Toxocara canis* and *T. mystax*. In both of these species, hemoglobin products could be extracted from the intestine.

Studies were negative with *Oxyuris equi* but the examination of material extracted with potassium hydroxide from the intestines of *Syngamus trachae*, *Schistosoma mattheei* and *S. mansoni* showed that the black pigment outlining the intestines could be reduced to a product with bands of a globin hemochromagen. Rogers concluded that *Strongylus edentatus*, *S. vulgaris*, *Schistosoma mattheei* and *S. mansoni* and *Syngamus trachae* were probably all blood-suckers. By calculation, he believed that it was impossible for the parasite to produce the amount of detectable hemochromagen and for the large molecule of hemoglobin to pass through the intestine. Thus, the products to be seen by the spectrophotometric method were derived from ingested blood and furthermore, the evidence suggested that these parasites digest hemoglobin. Since *Strongylus edentatus* was taking in enough blood to give a quantity of hemoglobin amounting to 1.56 per cent of the total weight and since hematin was found even in the extreme posterior end of the gut, it appears that blood is necessary to fulfill a definite physiological need of this parasite.

In another paper by Rogers in 1941, the digestion of protein by *Ascaris lumbricoides* and *Strongylus edentatus* was studied. The proteolytic enzymes and the comparative activity of these enzymes in the two parasites were compared. Peptic and tryptic digestion of extracts from the intestines of the worms were tested *in vitro* on gelatin, blood-albumin and casein during 6 days incubation at 37° C. The enzyme extracts were tested for their ability to produce amino acid nitrogen. With all three proteins, *Strongylus edentatus* was more active than *Ascaris lumbricoides* in digesting the same proteins *in vitro*, giving ratios ranging from 4.9 to 8.3, 12.5 to 40.9 and 2.6 to 4.7. Optimum tryptic digestion was obtained at pH 6.2. The ability of the proteases of *Ascaris* and *Strongylus* to digest oxyhemoglobin was tested also *in vitro*. Again enzyme extracts of *S. edentatus* were more active at the different levels of pH than similar extracts of *Ascaris*. Thus *Strongylus edentatus* is highly efficient in the digestion of protein when compared with *Ascaris lumbricoides*. This is in accordance with previous observations by Rogers (1941) that hydrolysis of protein is more rapid in parasites of the horse than in those of the pig, lending further weight to the suggestion that the first species consumes host tissue whereas the latter absorbs partially digested material from the lumen of the intestines. A possible explanation by Rogers of the large amount of blood ingested by *S. edentatus* and its high affinity for oxygen, is that a source of oxygen is needed and that this gas after being freed from oxyhemoglobin in the gut diffuses through the intestinal wall to the body cavities where it combines with the parasite hemoglobin and there becomes available for use in metabolic processes. Here again, data on the metabolic fate of the ingested blood plasma are needed.

Cestodes or tapeworms present an entirely different set of problems for studies of their metabolism and physiology. In the first place, the alimentary canal is absent and in the second place the protein content of these worms is less than the sum of glycogen and fat, lying somewhere between 30 and 40 per cent of their total body weight. Furthermore, there are few data to indicate the nature of the cestode protein. From available analyses, it seems likely that scleroproteins make up the primary nitrogenous content of these organisms. Keratin, elastin, collagen, reticulin, mucin, albumin and globulin have been identified in tissues of *Diphyllbothrium*

latum, *Taenia solium*, and *T. saginata*. The following by-products of protein metabolism have been identified both in adults and in larval stages of the tapeworm; ammonium salts, urea, creatinine, uric acid, acetylcholine, cystein, hexosamine; xanthine and guanine (reviewed by Smyth, 1947a).

Van Grembergen and Pennoit-de-Cooman (1944) compared the nitrogen metabolism of free-living planarians, with that of the parasites, *Fasciola hepatica*, *Moniezia benedeni* and *Taenia pisiformis* showing that the most important end-product was ammonia. Arginase was found for the first time in parasitic tapeworms but amino-acid oxidase and urease activity could not be detected.

Since the development of the *Cysticercus* at its final site in the host results from passage by way of the blood stream from the original site of infection, this organism and some consideration of its protein metabolism fits into the general theme of this paper. In studies attempting to analyse the 20 per cent total nitrogen content of *Cysticercus fasciolaris*, only 7 per cent was found to be protein on the basis of the precipitate obtainable by trichloroacetic acid (Salisbury and Anderson, 1939). Another worker, Eisenbrandt (1938) found that the non-protein fraction of the total nitrogen was surprisingly high. Since the *Cysticercus* includes a high volume of cystic fluid, the chemical composition of this fluid was found to be merely a transudate of the host serum. More accurate studies however, have shown that the vesicular and pericystic membranes had a composition very similar to that of blood but that the internal fluid contained less protein and more chloride than serum showing that selective permeability operates in the outer membrane.

This meager information about the protein metabolism of these very large parasites certainly indicates that many more studies are needed to explain what must be a very unique type of protein metabolism. Since methods have become available for the maintenance and growth of larval and adult stages of cestodes (Wilmoth, 1945; Smyth, 1947b; Hobson, 1948), it would be interesting to make use of some of the newer methods of chromatography for analyses of the protein to be found in the parasites themselves and in the suspending media used for *in vitro* maintenance.

The ability of cestodes to resist digestion by the gastric and intestinal juices of the host has been another phase of helminth physiology studied by a number of workers (Smyth, 1947a). The first theory, having to do with the production of antienzymes by the worm to resist digestion, could not be proven by experiment. The more plausible explanation is that the adult tapeworm is to be compared with an animal intestine, which is completely resistant to its own digestive juices while alive, but which autolyzes after death. This evidence suggests that antienzymes play no part in resistance to digestion but that the protective action of the cuticle is the sole protection for the living adult tapeworm. Even though proteolytic enzymes have been identified in *Taenia solium*, *T. saginata* and *Diphyllobothrium latum* and that the cuticle of *Cysticercus pisiformis* is impermeable to large molecules of proteins and peptones, current explanations for protein synthesis can still apply. Certainly the smaller molecular size of "building blocks" for protein synthesis should permit their diffusion through the cuticle in the same way that such building blocks pass through the red cell membrane for protein synthesis and growth of malarial parasites.

A comparison of the small amount of information available for the metabolism of nematodes and cestodes reveals very striking differences in the type of metabolism of these organisms. The field certainly needs additional exploration and the results

should be of value in an attempt to determine the basis for the pathogenicity of these organisms to their host. When one realizes that cestodes have a pH tolerance range of approximately 4 to 11 and that helminths in general have to adapt themselves to a wide range of environmental conditions, this information should be particularly useful in developing better methods for *in vitro* maintenance and study of these diverse parasites.

Protozoa

In passing on to the protozoa, numerous pathogenic and non-pathogenic species that live either in plasma or cells of blood at sometime during infection in their respective hosts, are available for *in vitro* study. A number of organisms such as *Plasmodium knowlesi* in monkeys, *P. gallinaceum* in certain avian hosts, and species of pathogenic trypanosomes in experimental animals produce heavy infections needed for biochemical studies. A number of blood protozoa can be maintained or cultivated *in vitro* free of bacteria or other cells for periods that permit analysis of factors for survival, growth and metabolism. Problems of the identity of essential growth factors, metabolic behavior, mode of action of drugs and development of drug resistance can be studied with these parasites under controlled conditions free of the complicating factors governing the infection in the host.

Scattered experiments and observations on protein metabolism have been carried out for a variety of parasitic protozoa but the most complete evidence has been obtained from *in vivo* and *in vitro* studies with plasmodia. Conventional manometric and biochemical methods have been utilized for short and long term analytical studies. Cultural experiments to study the nutrition of the organisms have been extensive, but the majority of methods and basal media used have not yet approached the degree of accuracy achieved in studies of bacterial metabolism and essential for the interpretation of results in terms of detailed metabolic pathways.

The early studies of Salle and Schmidt (1928) and Salle (1931), with the metabolism of *Leishmania tropica* and *L. donovani* grown in cultures, revealed that carbohydrate excited a marked sparing action on the protein of the medium. The proteolytic action of the organisms was increased in the absence of dextrose, a fact that demonstrated a preference for dextrose as a source of energy. The utilization of proteins was demonstrated also by an increase of split protein products. Studies of a similar type with the aflagellated stages of these parasites are needed to interpret their metabolism and pathogenesis in reticulo-endothelial cells.

Although species of *Leishmania* can be grown easily in cultures containing blood from different animals, no serious attempt has been made to analyze the specific protein nutrients needed for their growth and multiplication. The majority of the studies have been concerned with enriching media to enhance growth. Lwoff (1940) has shown the need for hemin and ascorbic acid in cultures of certain species of *Leishmania* and *Trypanosoma cruzi* in a manner similar to *Hemophilus* sp. The further study of the growth requirements of *Hemophilus* showed that growth factor or hemin was needed only under aerobic conditions but no similar studies have been done with *Leishmania*. It is now generally agreed (Porter, 1946) that hemin under aerobic conditions functions by being synthesized into several enzymes (catalase, peroxidase, cytochrome oxidase) or carrier substances (cytochromes).

Studies with the protein metabolism of trypanosomes seem to have been neglected

in deference to the emphasis placed on carbohydrate metabolism and the effect of drugs on this cell function. Present knowledge of coupled biochemical reactions in the cell should lead us to reexamine the dictum of over a decade ago that protein metabolism of these pathogens is relatively unimportant (von Brand, 1938). The necessity for protein degradation products *in vivo* or *in vitro* to supply the "building blocks" for growth and multiplication could hold the key to the discovery not only of essential metabolites but also to the finding of analogues that could inhibit growth. Krijgsman (1936) identified cathepsin, carboxyl-peptidase, amino polypeptidase and dipeptidase in *Trypanosoma evansi* indicating that proteins play an essential part in the metabolic functions of trypanosomes. The conclusion by Schueler (1947) that drug resistance acquired by trypanosomes may involve a shift in the isoelectric points of some of the constituent proteins of the trypanosomes bears out the importance of exploring the protein metabolism of these blood parasites.

Available information about the protein metabolism of the asexual stages of malarial parasites has resulted from short term biochemical experiments and from studies on factors required for *in vitro* survival and cultivation. These combined studies have shown that the asexual stages of plasmodia, responsible for production of clinical malaria, degrade the proteins of the red cell and synthesize their own specific proteins with the production of characteristic pigment found in malarial parasites. The utilization of red cell proteins and the diffusion of nutrients from the plasma through the red cell membrane leads to growth and production of a normal number of progeny needed for multiplication by invasion of new host cells. Growth and multiplication involves coupled reactions of carbohydrate and protein metabolism and enzyme activity to produce synthesis of cytoplasm.

In 1911, Brown showed that the hemoglobin of the red cell was split by plasmodia to form pigment or hemozoin and globin in the parasite. The belief resulting from early investigation that hemozoin is hematin has been confirmed. Ghosh and Nath (1934) found that the iron, carbon and hydrogen values for the malarial pigment derived from *P. knowlesi* agreed with those of hematin. Huff and Bloom (1935) and Huff and Coulston (1944) have shown that the exoerythrocytic stages of *P. elongatum* and *P. gallinaceum* in non-hemoglobin containing cells produce no pigment. Morrison and Williams (1941), Morrison and Anderson (1942) and Anderson and Morrison (1942) studied the characteristics of malarial pigment and the role of the pigment in the production of disease. More recently, Rimington *et al* (1947) extended chemical studies of malarial pigment and identified the pigment from *P. knowlesi* and *P. gallinaceum* as hematin. The need for globin by plasmodia during growth within the erythrocyte requires the splitting of large quantities of hemoglobin. Ball *et al* (1948) presented data indicating a sizeable amount of the hemoglobin being split to form hematin during growth of *P. knowlesi*, *P. vivax* and *P. lophurae* in red blood cells. Morrison and Jesky (1948) confirmed this finding with *P. knowlesi*, arriving at a calculated value of 76 per cent of the hemoglobin in the host cell being destroyed by the growing parasite. Moulder and Evans (1946) working with *P. gallinaceum* and Morrison and Jeskey (1947, 1948) working with *P. knowlesi* have shown that the protein of the red cell is broken down by hydrolysis or phosphorylysis and approximately half of the amino acids are utilized by the plasmodium for the synthesis of protein, while the remainder diffuses out of the erythrocyte. The first group of workers found that chicken erythrocytes infected

with *P. gallinaceum* produced large amounts of amino nitrogen (amino acids and peptides) when incubated in air and in the presence of glucose. When glucose was absent, much of the amino nitrogen appeared as ammonia. However, when oxygen was absent, even in the presence of glucose, there was little splitting of the protein to produce amino nitrogen and nearly half of the liberated nitrogen was ammonia nitrogen. Although *P. gallinaceum* can deaminate amino acids, this work indicates that the normal course of events for growth and reproduction of the parasites is the utilization of amino acids for protein synthesis. Probably only small amounts of amino acids are consumed in oxidative processes since the respiratory stimulation of *P. knowlesi* is very small when amino acids are added in Warburg respirometer experiments (McKee *et al*, 1946). The interpretation of all these results is dependent on the conditions of the experiments, since the parasites being studied were suspended in balanced salt solution with adequate glucose but deficient in diffusible building blocks for protein synthesis.

In spite of the apparent excess of amino acids produced by the metabolism of the parasite, cultural and respiration experiments have shown that methionine must be added to the medium because it is an essential building block for the growth of *P. knowlesi* (McKee *et al*, 1947). The explanation for this requirement probably lies in the fact that hemoglobin contains only about one per cent methionine while most proteins contain 3 or 4 per cent of this amino acid. This need for extra methionine has been demonstrated for *P. knowlesi* both *in vivo* and *in vitro* (McKee and Geiman, 1948) since monkeys infected with this parasite require methionine in their diet for growth and multiplication of the plasmodium. As stated previously (Geiman, 1948) these studies have shown that asexual stages of plasmodia are not true intracellular parasites capable of living entirely on the cytoplasm of the host cell, but that they need to obtain from the plasma other substances such as glucose, methionine and certain vitamins, purines and pyrimidines in order to achieve normal growth and multiplication.

Evidence is accumulating about the relationships between vitamins, enzymes, hormones and amino acids in the metabolism of living cells (Mitchell, 1943; Jensen and Tenenbaum, 1944; Knight, 1945 and Woods, 1948). The rôle of these substances in growth and multiplication and hence protein metabolism of plasmodia has not been completely explored. In our *in vitro* cultural studies (Geiman *et al*, 1946; Anfinsen *et al*, 1946 and Geiman and McKee, (unpublished results)) and *in vivo* nutritional studies (McKee and Geiman, 1946, 1948 and Geiman and McKee, 1948) effects on growth and protein synthesis were noted in numerous experiments. Under certain experimental conditions, the parasites fail to achieve normal size, cytoplasm and chromatin show degenerative changes, there is poor pigment production and the number of merozoites is reduced.

The first encouraging lead for *in vitro* growth and multiplication of *P. knowlesi* was obtained when proteose peptone (Difco) was added to our culture medium. Later, para-aminobenzoic acid (PABA) was found to be the growth promoting substance in the peptone and an essential metabolite for *in vitro* multiplication of *P. knowlesi*, the parasites requiring a concentration of 2–10 γ per liter. PABA according to Ansbacher (1944) is considered as a relative to the vitamin B complex in its growth promoting functions. Certainly the addition of this small amount of PABA to the medium at this stage of our studies made the difference between success and

failure. The results with methionine have been discussed above and the importance of the vitamin block and the purine and pyrimidine block to cultivation *in vitro* have been discussed elsewhere (Anfinsen *et al.*, 1946).

The evidence for the synthesis of enzyme proteins in malarial parasites is indirect. Christophers and Fulton (1938, 1939), Fulton (1939), Coggeshall (1940), Maier and Coggeshall (1941), Coggeshall and Maier (1941), Velick (1942) and McKee, *et al.* (1946) have shown increases in the utilization of glucose and the consumption of oxygen by malarial parasites. Velick (1942) studying *P. cathermerium* obtained a slow increase in the oxygen uptake as the parasite grew and then a greatly accelerated respiration when nuclear division began. A simultaneous increase in the cytochrome oxidase activity was detected. McKee *et al.* (1946) determined a two and one-half fold increase in the lactic dehydrogenase content of red cells infected with *P. knowlesi*. Ball *et al.* (1948) showed a 6 to 15 fold increase in the flavine-adenine dinucleotide content of *P. knowlesi*. This compound is the coenzyme for several oxidative enzymes. The amount of increase for both of these enzyme constituents is determined by the degree of development of the parasites.

The fact that cells engaged in protein synthesis have a high pentose-nucleic acid content is generally accepted from experimental evidence (Davidson, 1949). The importance of nucleoproteins to the metabolism of plasmodia has been deduced from observations on the rapid increase of nuclear material during schizogony of asexual stages and during the differentiation of sporozoites in oocysts. The formation of nucleoproteins has not been studied directly, but Ball *et al.* (1948) by phosphorus analyses of *P. knowlesi* obtained evidence for the synthesis of nucleic acids. Calculations from the phosphorus data indicate an increase in the quantity of nucleic acids during growth and the formation of 5.46 gm. of nucleic acids per 5×10^{12} parasitized cells in approximately 1000 gm. of packed parasitized cells. These calculations are based on the assumption that no phosphoprotein is present but this may be incorrect. However, these results show that the parasites are capable of synthesizing their own nucleic acids from simpler compounds and that purines and pyrimidines added to culture media gave enhanced growth and multiplication. In fact, by calculation from the analyses (Ball *et al.*, 1948), our original medium was shown to be deficient in the quantity of purine and pyrimidines needed to give normal growth and multiplication. Results with the Feulgen nuclear staining reaction with *P. knowlesi* and *P. vivax* show that nucleic acids are present in these parasites. Furthermore the intensity of reaction increases with the age of the parasites, indicating the synthesis and accumulation of nucleic acids (Deane, 1945).

The importance of protein metabolism to malarial parasites has been shown elsewhere in a schematic diagram (Geiman, 1948). This phase of metabolism is coupled with carbohydrate and lipid metabolism, all of which are necessary for enzymatic function, growth and multiplication. The similarity of these metabolic processes for species of plasmodia other than *P. knowlesi* and *P. gallinaceum* are not known. Differences might be of degree and therefore of actual importance to the persistence of specific characteristics and the potential pathogenesis to susceptible hosts. The importance of blood proteins and their metabolism to the development of the cycle in female anopheline mosquitoes is not known but hereditary factors are involved (Huff, 1941). One can predict however, with some degree of certainty, that the differential susceptibility of species of anopheline mosquitoes for mammalian

and avian plasmodia are linked not only to hereditary factors but also to biochemical differences of the ingested blood, to the metabolic and nutritional requirements of the plasmodia, and to the physiology of the female mosquito (Hovanitz, 1947).

CONCLUSIONS

Thus we see that parasite organization is as much an organization of processes as of structures. The time has come for the processes to be more widely studied. The meager information available on protein metabolism of parasites is insufficient to establish a pattern. Descriptive parasitology has been built on a solid foundation, but physiological parasitology has been sadly neglected, lagging far behind advances in the related field of bacteriology (Porter, 1946) obtained by applying the modern tools of physiology, biochemistry, biophysics and pharmacology. On the one hand the parasitologists have not been adequately trained in these fields, and on the other hand skilled investigators in preclinical medical sciences are largely unaware of the wealth of material available in parasitology for study.

Two alternatives are open to us in training parasitologists to meet the demands for critical research in physiological parasitology. In training future parasitologists, we must shift our emphasis from the morphological to the physiological. In doing this, opportunities for graduate study in biochemistry, pathology, medical bacteriology, and pharmacology must be provided. Otherwise, we can only hope, as an alternative, to interest students trained in those fields to collaborate with us in exploring the extensive field of physiological parasitology.

We have heard in this symposium a review of milestones in our knowledge of the physiology of parasites. The obvious conclusion as with many branches of science is that our knowledge needs amplification. In closing then, the following question is asked, what can you do as parasitologists, to help solve the many riddles about the parasitic mode of life that remain to be answered?

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ON THE ESTIMATION OF *TRICHURIS* WORM BURDENS IN PATIENTS

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INTRODUCTION

Various workers have endeavored to determine the number of ova produced by female *Trichuris trichiura*, so that by means of egg counts one can estimate the number of worms harbored. Probably the first attempt was made by Leuckart (quoted by Moosbrugger, 1891), who estimated the daily output to be 1000 ova per female. Moosbrugger (1891) concluded, from one postmortem study of an 18 month old boy, that each female laid approximately 3333 eggs per day. Manalang (1928), using cadavers, estimated that in normal cases 310 ova per gram represented one female worm, whereas in cases with intestinal pathology 669 ova per gram were produced by each female. Corrêa and Mellone (1938) reported on the results they obtained from the examination of 19 postmortem cases, and their figure of 315 ova per gram per female corresponded closely with that given by Manalang.

In experiments conducted on dogs, using *T. vulpis*, Miller (1939, 1941) found that each female of *T. vulpis* produced about 2035 ova per day, and he concluded that Leuckart and Moosbrugger were more nearly correct in their estimation of the daily output per female than were Manalang or Corrêa and Mellone. He qualified his statement by admitting that the egg production of *T. vulpis* may not be of the same order as that of *T. trichiura*.

Inasmuch as efficient drugs have not been available in the past for *Trichuris* infections in most countries, determinations of the number of ova per gram per female have not been practical on living patients. This problem has been solved to some extent by the use of enteric-sealed tablets of emetine hydrochloride (Burrows, Morehouse and Freed, 1947), which caused the elimination of most of the *Trichuris* harbored. The present paper reports the results obtained from nine of the patients treated with this drug.

METHODS

Of the thirteen patients used in a previous treatment program (Burrows, Morehouse and Freed, 1947), who had a careful search made for all *Trichuris* passed during treatment, nine were used in the present study. Four were eliminated for the following reasons: no worms were recovered from one; only one *Trichuris* was recovered from another who showed a high egg count both before and after treatment; another passed comparatively few worms for one with a high egg count, thus giving a disproportionately high number of eggs per gram per female; and the other passed many fragmented worms after being given a saline purgative to end a constipated period of several days.

In preparation for treatment of these patients three to six fecal specimens were obtained from each. Using the Stoll method at least two egg counts were made of

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each specimen. The correction factors used were those suggested by Brown and Otto (1941). However, in some specimens the character of the stool or the presence of large particles of undigested food made it necessary to use some intermediate factors, such as, 1.25, 1.75, 2.5 etc.

After treatment the egg counts were continued for some time, but only the first two post-treatment counts were used, by averaging them, in determining the number of eggs lost by each patient. Four of the nine patients showed no *Trichuris ova* in their stools for two to four weeks after treatment. All counts were made by the writer in order to eliminate variations in technique, which may occur when specimens are counted by different persons.

In the previous paper (Burrows, Morehouse and Freed, 1947) the three pre-treatment egg counts of each patient, which were more nearly alike, were selected for the pre-treatment average. In the present paper all pre-treatment counts are used. Therefore, the averages vary somewhat from those of the other paper.

The worms from these patients were separated by sex and counted to determine the exact number of each sex passed. During this more careful count; several additional worms, which were overlooked in the previous work, were teased out of fragments of mucosa, thus increasing slightly the counts on five patients.

In an effort to seek some correlation between the number of ova per gram per female and the size of the females harbored, all female worms from those having passed few worms and a representative number of worms from those who passed large numbers were measured. This was done by projecting the image of the worm to a magnification of eight times, tracing the outline on paper, measuring the tracing and converting back to millimeters. So far as possible the entire worm was traced, but in many the slender anterior portion was so coiled that only the "handle" (robust posterior end) could be traced with accuracy. In all, 201 worms were traced in their entirety and in 186 other worms the "handle" alone was traced and measured.

RESULTS

Sex Ratio of Trichuris Recovered

A total of 4,582 worms were recovered from the nine patients, the number of worms per person ranging from 14 to 2,544. Of the total number of worms passed, 2,477, or 54.0 per cent, were females, which gave a ratio of 1.00:1.17. Only one patient (B. C.) passed more males than females and the difference was slight (Table 1, Section D).

Relation of Number of Female Trichuris Recovered to Number of Ova per Gram

The average of the first two post-treatment egg counts of each patient was taken and subtracted from the average of the pre-treatment egg counts in order to determine the reduction in number of eggs as a result of treatment (Table 1, Sections A-C). This was done both for the actual count and for the count corrected according to the values recommended by Brown and Otto (1941) and others. Inasmuch as some (Scott and Headlee, 1938; and others) consider the corrected values to be rather high, both sets of figures are given.

It is evident (Table 1, Sections E, F) that there was a wide range in number of ova per gram per female and per worm. The average for the actual counts was 154 and for the corrected counts 225 ova per gram per female, so that the true figure probably falls somewhere between these two. For total number of worms

passed the number of ova per gram per worm probably lies somewhere between the actual count average of 87 and the corrected count average of 127. If the corrected values for egg counts be considered slightly high, it may be assumed that the number of ova per gram per female is about 215 and the number per gram per worm is about 120.

Relation of Size of Female Trichuris to Number of Ova per Gram

In the measurements of the entire length of 201 female worms, it was found that the average percentage of "handle" to total length was 33.1. Inasmuch as this is

TABLE 1.—*Experimental data. (Egg counts in sections A, B and C are given in thousands of ova per gram of stool)*

Patient		H.M.B.	B.C.	S.K.C.	R.M.F.	A.M.G.	M.I.	B.N.	E.P.	W.T.
A. Pre-treatment Egg Counts										
1st	A*	14.7	10.5	0.2	31.4	7.0	7.9	6.6	12.9	12.7
	C	22.0	15.8	0.8	54.0	7.0	11.8	13.2	19.4	50.8
2nd	A	8.2	14.6	0.3	52.4	9.9	17.2	15.9	9.3	70.6
	C	16.3	18.2	0.6	91.8	9.9	21.4	23.9	16.4	141.2
3rd	A	16.7	10.0	0.8	76.8	3.6	16.1	16.5	10.1	47.3
	C	20.9	15.0	1.0	115.1	7.2	24.2	33.0	15.1	94.6
4th	A			1.4	91.1			9.9		58.6
	C			2.0	91.1			24.7		102.6
5th	A							67.4		
	C							110.4		
6th	A							51.4		
	C							128.5		
Aver.	A	13.2	11.7	0.7	62.9	6.8	13.7	28.0	10.8	47.3
	C	19.7	16.3	1.1	88.0	8.0	19.1	55.6	17.0	97.3
B. Post-treatment Egg Counts										
1st	A	2.5	1.5	0	4.0	0	1.0	0.5	0	0
	C	2.5	2.3	0	5.0	0	2.4	0.9	0	0
2nd	A	1.2	1.9	0	5.1	0	3.0	0.2	0	0
	C	1.4	2.8	0	6.4	0	3.0	0.4	0	0
Aver.	A	1.8	1.7	0	4.6	0	2.0	0.3	0	0
	C	2.0	2.6	0	5.7	0	2.7	0.7	0	0
C. Egg Loss per Gram (Pre-treatment Aver. Less Post-treatment Aver.)										
	A	11.4	10.0	0.7	58.3	6.8	11.7	27.7	10.8	47.3
	C	17.7	13.7	1.1	82.3	8.0	16.4	54.9	17.0	97.3
D. Number of Worms Recovered										
♂♂		28	88	6	313	23	24	230	206	1187
		43	81	8	327	31	33	337	260	1357
Total		71	169	14	640	54	57	567	466	2544
E. Average Number of Ova per Gram per Female**										
	A	265	123	88	178	219	355	82	42	35
	C	412	169	135	252	258	497	163	65	72
F. Average Number of Ova per Gram per Worm***										
	A	161	59	50	91	126	205	49	23	19
	C	249	81	79	129	148	288	97	36	38

* A = Actual Count; C = Corrected Count.

** Actual count average is 154; corrected count average is 225.

*** Actual count average is 87; corrected count average is 127.

almost exactly one-third, the length of those worms, in which the "handle" only could be measured, was computed by multiplying by three. Table 2 is arranged in order of size of worms, rather than in alphabetical order as in the other table.

This table shows that, to some extent, the size of worms harbored can be correlated with the number of ova per gram per female, in that the larger the worms the greater the egg production. The major discrepancy in the table concerns one patient (M. I.). Inasmuch as many of the worms passed by these patients during

treatment were coiled up in sloughed off mucosa, there is a possibility that some worms remained attached to the mucosa in the intestine after death and gradually disintegrated before being passed out in the feces some days later. Such may have been the reason M. I. showed a large count with comparatively few, small worms passed.

DISCUSSION

In the present study 54.0 per cent of the total worms recovered were females. This is a ratio of 1.17 females to each male. This ratio agrees rather closely with the figures found by Moosbrugger (1891), who found 50.3 per cent females (ratio of 1:1.01) and by Manalang (1928), with 56.3 per cent females (ratio of 1:1.28). Corrêa and Mellone (1938) and Miller (1941) both reported the ratio to be 1:2, or 66.7 per cent females.

From the evidence presented herein it appears that the average number of ova per gram per female *Trichuris* is about 215. This figure is approximately two-thirds of the 310 ova per gram per female recorded by Manalang (1928) and the 315 reported by Corrêa and Mellone (1938). Due to the different methods probably used by Leukart and by Moosbrugger, a definite comparison would be difficult. However, the egg production found for *T. trichiura* appears to be much higher than that reported for *T. vulpis* (Miller 1939, 1941).

TABLE 2.—Relation of Size of Female *Trichuris* to Number of Ova per Gram

Patient	Aver. Length of Females (in mm.)	No. Ova per Gram per Female (Corrected Count)
H.M.B.	41.4	413
R.M.F.	36.6	252
A.M.G.	36.3	259
B.C.	36.3	170
B.N.	34.8	163
S.K.C.	30.3	138
W.T.	30.3	72
M.I.	29.4	498
E.P.	29.4	65

Although the average number of ova per gram female was found by Manalang, Corrêa and Mellone and the writer to be between 215 and 315, a careful examination of the individual cases reveals a wide range of figures. As a result, it is easier to estimate the number of worms harbored by a group of patients than it is to estimate the number harbored by any given patient.

Miller (1941) stated that the egg production per female in light infections was higher than in heavy infections. In the present study no consistent correlation could be found from that standpoint. However, the average number of ova per gram per female was found to be 144 in the five patients harboring over 100 worms each and to be 327 in the four patients having less than 100 worms each.

There was a rather striking correlation between the number of ova per gram per female and the size of the females harbored. With one major exception, the larger the average size of the females harbored the greater the egg production per worm. As far as the writer could determine, this phase had not been reported previously.

Although the average number of ova per gram per female probably lies somewhere between 200 and 300, any estimation of the *Trichuris* worm burden of a given patient is subject to variation due to three factors: (1) the size of the worms

harbored; (2) the intensity of the infection, whether light or heavy; and (3) the age of the infection. A fourth factor which may be included is that of stool size, which influences the egg counts and, consequently, the estimate of the number of worms harbored (Stoll, 1924). However, if a series of pre-treatment specimens are examined, the variations in egg counts are smoothed out and this factor becomes less important than the first three mentioned factors.

If the infection were comparatively recent, the egg production would be lower than in an older infection and the egg counts would show a definite increase over a period of time (Miller, 1941; Burrows, Morehouse and Freed, 1947), so that a single count made during the increase would not be valid. If a person were exposed constantly to new infections, as are many mental patients, due to their untidy habits, one should estimate the number of ova per gram per female to be much less than 300. On the other hand, if the infection were a light one and old enough for all worms to have matured, then the number of ova per gram per female might be estimated at more than 300.

SUMMARY

1. Of the 4,582 *Trichuris* recovered from nine patients, 54.0 per cent were females, giving a ratio of 1:1.17.
2. The average number of ova per gram per female was found to be about 215 and per worm about 120.
3. A positive correlation between the size of the female worms and the number of ova per gram per female and a negative correlation between the intensity of the infection and the number per gram per female were found.
4. Any estimation of the number of worms harbored by a given patient would, in all likelihood, be incorrect, due to these factors, which probably would be unknown to the investigator, namely: the age of the infection, the intensity of the infection, the size of the worms harbored, and, if only one or two specimens were counted, the size of the normal stool.

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PARASITOLOGICAL STUDY ON THE ESKIMOS IN THE BETHEL AREA OF ALASKA

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During the summer of 1949 a study was conducted to determine the incidence of intestinal parasites and trichinae in the Eskimos in the Bethel area of Alaska. The patients examined came to the Alaska Native Service Hospital and Clinic at Bethel from the lower Kuskokwim River region. The majority were contacted through the out-patient general clinic. They were Eskimos of all ages, nonselected, consecutive patients who were physically able and willing to return daily to the hospital.

An attempt was made to obtain six consecutive fecal samples, six perianal pinworm swabs and trichina skin test each individual studied.

Each fecal sample was examined by using a direct wet saline smear, modified zinc sulfate centrifugal flotation concentration procedure with iodine smear and modified sodium sulfate-triton-ether procedure (Faust and Ingalls, 1946) and screening through 10 and 40 mesh sieves.

Perianal pinworm swabs (Jacobs, 1942) were taken on patients of all ages. The in-patients were swabbed in the morning before a bath or bowel movement. The out-patients were examined in the morning between nine and eleven o'clock when they brought their fecal samples. Swabs were observed until eggs were found or until six slides per person were examined.

On the trichina intradermal skin tests, half hour readings were made and 24 hour when possible.

From 2 to 6 fecals per person were examined on the 100 Eskimos observed. Six stools were observed on 82 persons, 5 on 2 persons, 4 on 4 persons, 3 on 7 persons and 2 on 5 persons, making a total of 549 stools examined or an average of 5.49 stools per person. As a group the Eskimos were very cooperative. Of the 118 persons asked to bring in six stools, only 11 did not return, 7 brought in only one stool and 100 brought in from 2 to 6 specimens.

The incidence of the intestinal protozoa (table 1) is similar to that of the general population in the northern United States except for the absence of *Chilomastix* and *Trichomonas* and the lower rate of infection with *E. histolytica*.

Diphyllobothrium was the only cestode infection observed (table 1). In only one of the 15 persons were segments passed in the stool.

Brown, et al., 1948, in the examination of one fecal sample per person found 22 cases of *Enterobius vermicularis*, 6 cases of *Diphyllobothrium*, one infection of *E. coli* and one of *Giardia lamblia* in 33 Eskimos on Southampton Island.

Pin worms, *Enterobius vermicularis*, were the only human intestinal nematode infections observed in examining 101 Eskimos. The incidence (table 1) may be

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the minimum infected number because most of the swabs were not taken at the most advantageous time. In the age group 1–20 years, 49 people were examined with 24 (49%) positive. Of the 52 people in the age group 21–70 years, 35 (67%) were infected. Of the 40 males, ages 1–70 years, 20 (50%) were positive, and of the 61 females in the same age group 39 (64%) were infected. No adult pinworms were observed by any method used and in only two stools from 59 pinworm infected people, were eggs recovered and then by the zinc sulfate concentration procedure.

The ascarid larvae (table 1) were identified by Mr. J. T. Lucker in the laboratory of Dr. G. Dikmans, Zoological Division, Agricultural Research Center, Beltsville, Maryland. Dr. Dikmans reports as follows: "All appeared to be ascarid larvae, that is, larvae of some nematode belonging to the family ASCARIDIDAE. The theoretical possibilities are (1) that the worms are larvae of *Ascaris lumbricoides* eliminated spontaneously as a result of the development of host resistance or following the use of an anthelmintic, (2) that they are ANISAKINAE larvae ingested with the flesh of fish and passed from the digestive tract because man is an unsuitable host or because cooking, or freezing, has impaired their viability. One specimen,

TABLE 1.—Incidence of Parasites in the Eskimos

Parasite	Percentage Infected
<i>Endamoeba histolytica</i>	3
<i>E. coli</i>	51
<i>Endolimax nana</i>	8
<i>Iodamoeba bütschlii</i>	4
<i>Giardia lamblia</i>	1
<i>Diphyllobothrium</i> *	15
<i>Enterobius vermicularis</i>	58
<i>Trichinella spiralis</i>	6
Ascarid larvae	10

* Species to be studied by Dr. L. J. Thomas, University of Illinois.

however, was definitely identifiable to the subfamily ANISAKINAE, probably to the genus *Porrocaecum*, and another could, with reasonable certainty be determined as a member of the genus *Anisakis*." It would seem that the latter possibility is most likely correct as no *Ascaris lumbricoides* eggs or adults were observed.

The eggs of *Ascaris*, *Trichuris*, *Necator*, or trematodes, were not detected although the procedures were sufficient for their recovery. Climatic conditions should not hinder the presence of the first two in humans as they were found in dogs in the area.

Of the 100 Eskimos from whom fecal samples were examined, 39% showed mixed parasitic infections. The most common being 16% for *E. coli* and *E. vermicularis*.

Intradermal skin tests were made on 150 Eskimos, ages 4–74 years, revealing an incidence of 6.6% trichina. Of the 10 positives, 4 gave a history of having eaten frozen bear meat, one raw seal and whale liver, one raw whale, one pork and 3 gave no history of eating raw meat.

In the complete study 192 Eskimos were examined by one procedure or all. They came from 52 villages as far as 240 miles north, 240 miles northeast, 160 miles west and 190 miles south of Bethel. There was no apparent geographic localization of any of the parasitic infections.

The author wishes to thank the U. S. Public Health Service, Anchorage, Alaska

and the medical staff of the Alaska Native Service Hospital, Bethel, Alaska for their cooperation in this study.

SUMMARY

1. The incidence of the intestinal protozoa in the Eskimos of the Bethel area is similar to that of the general population in the northern United States.
2. *Diphyllobothrium* sp. was observed in 15% of the population examined.
3. *Enterobius vermicularis* occurred in 58% of the people, being most common in the age group 21-70 years.
4. Mixed intestinal parasite infections occurred in 39% of the people.
5. The intradermal trichina skin test showed 6.6% of the population to be reactors.

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A LETHAL TRAP FOR CAPTURING SMALL MAMMALS WITH THEIR ECTOPARASITES

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In past attempts to determine the abundance and species of ectoparasites normally present on small roving mammals, the collector usually obtained the hosts by killing or by live trapping. In both cases, the conclusions drawn concerning the relative abundance of ectoparasitic species were subject to error since unattached ectoparasites are lost from freshly killed animals in proportion to the degree of violence used in killing², and live-trapped animals may lose, or even gain, ectoparasites prior to being bagged.

In order to avoid these sources of error, a lethal gas trap has been designed which, upon being sprung, kills the trapped animal and all of its ectoparasites by means of a hydrogen cyanide gas. A true count is thus permitted of the number and species of ectoparasites on the animal at the time it was caught. The trap has been used successfully under field conditions in capturing the rice rat, *Oryzomys palustris*; the cotton rat, *Sigmodon hispidus*; the deer mouse, *Peromyscus leucopus*; and the domestic roof rat, *Rattus rattus*. A larger model has been used successfully to catch the raccoon, *Procyon lotor*.

The construction details of this trap are presented in figure 1. The design is such that it may be built in any field shop possessing the usual equipment. The box is constructed of marine plywood to withstand all weather conditions and yet remain relatively gastight. The current cost of the materials from which the trap is constructed is estimated at \$2.82.

The improved trigger mechanism and door release (fig. 1, details B, C) offer ease in setting and remarkable stability under ordinary conditions; yet, they retain the necessary sensitivity to render the arrangement practical. Under windy conditions, however, a tendency for the aluminum door to blow shut has been noted. This difficulty may be overcome by increasing the length of the arms of the U-shaped door release and by increasing the height of the two supporting screw-eyes, thus giving greater stability to the door.

The lethal element is provided by Cyanogas A-Dust³, which is contained in a .410 gauge shotgun shell. The shell, inserted in a 3/8-inch iron pipe mounted in the top of the box (fig. 1, detail D), is exploded by a hammer attached to the door (detail A). The shells containing the Cyanogas A-Dust may be hand loaded, but care must be taken to seal the chamber containing the dust in order to prevent deterioration of the primer and premature generation of the gas.

To load a shell with Cyanogas A-Dust, first remove the terminal wad and shot from a standard shotgun shell, but leave the powder and protecting wads

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¹ From Technical Development Division, Savannah, Ga.

² Unpublished data.

³ A product of American Cyanamid & Chemical Corp., New York, N. Y. The trade names are used as a means of identifying products under discussion, and do not represent endorsement of the products by the Public Health Service.

of the shell. Finally, seal the terminal end with paraffin to prevent generation and loss of the gas prior to release of the dust from the exploded shell. The explosive force of the primer is sufficient to blow dust and wads from the shell. If properly sealed, the life of a Cyanogas-loaded shell is at least 2 months; but improperly sealed shells may deteriorate and fail to fire after a very few days.

In tests with laboratory white rats which had been infested artificially with the rat flea, *Xenopsylla cheopis*, and the rat mites, *Liponyssus bacoti* and *Echinolaelaps echidninus*, all ectoparasites and the rats were killed in less than 1½ minutes after the traps were sprung. All activity on the part of the rats ceased within 30 seconds. These tests were performed at temperatures of approximately 90° F.

Recovery of the dead ectoparasites is best accomplished by returning the traps containing catches to the laboratory where the animals may be combed for the removal of ectoparasites and where those ectoparasites not on the animals may be separated from the spent Cyanogas dust. Most of the ectoparasites, however, will be found still buried in the fur of their hosts.

In a series of tests performed under natural conditions at the Savannah River Wildlife Refuge during December of 1948, the lethal gas trap was found to function very efficiently in capturing the eastern rice rat, *Oryzomys palustris palustris*, which was the predominant rodent present in the area trapped during that period. Out of a total of 47 trap-nights, 13 rice rats were caught and killed giving a trap yield of 28 percent. However, during the test period, the traps were frequently tripped by cattle or robbed and tripped by raccoons, so that the efficiency actually might have been greater than indicated.

In March and April of 1949, eight of the lethal gas traps were tested in the same general area in comparison with eight rabbit box traps as modified by Richter and Emlen (1945), and eight No. 2 Humane Havahart traps⁴. The traps were set in an alternating fashion along approximately a half mile of dike so as to avoid favoring any one type of trap. Each type was in use during a total of 111 trap-nights. During this period, a total of 12 animals (7 rice rats, 2 cotton rats, and 3 deer mice) was caught in the lethal gas traps, giving an effective trap yield of 11 percent. The results with the modified rabbit box traps were almost equally as good with 11 animals caught (6 rice rats, 2 cotton rats, and 3 deer mice). The Humane Havahart traps yielded 7 animals consisting of 5 rice rats and 2 cotton rats, but in fairness to this trap, it must be stated that the size used in this test was not intended to hold mice.

SUMMARY

A lethal gas trap employing hydrogen cyanide gas as the killing agent is described. Construction details are given. This trap has been used successfully to obtain information concerning the abundance of ectoparasites on roving small animals. Performance data under field conditions are presented.

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⁴ A product of Allcock Manufacturing Company, Ossining, N. Y.

SOME SOURCES OF VARIABILITY IN CULTURES OF ENTOZOIC AMOEBAE. I. THE EFFECT OF AGE OF CULTURE, SIZE OF INOCULUM AND AMOUNT OF STARCH.*

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The variability of living organisms is particularly apparent in cultures of micro-organisms where each population is part of a microcosm more or less under the control of the investigator. In those circumstances where the living elements of the microcosm are univariate, as in the case of pure cultures of bacteria or protozoa, conditions are optimum for assay of environmental effects. Most protozoa, and particularly the entozoic amoebae, have thus far been obligately symbiotic in multivariate microcosms where environmental changes may affect them indirectly through the associated organisms. Regardless of the ultimate effect, particular causes can be identified only if they result in significant differences in yield.

While variability is the rule, it can be brought to a minimum by bringing under control those factors responsible for the excess. Presumptively these factors may be numerous, but in practice many of them are constant in behavior and do not contribute to excess variation. In the case of the multivariate microcosm the bacterial flora will have a stable behavior in the quantitative sense as was shown by Clinkston (1948), and so will many cultures of free-living protozoa (cf. Richards 1941). On the other hand cultures of entozoic amoebae have proven so extremely variable as to defy evaluation of any save the crudest effects. In the only available report of a study of the population curve of *Endamoeba histolytica* Balamuth and Howard (1946) recognized the apparently inescapable variability involved and for that reason were unwilling to make specific claims as to the absolute values of particular data.

Given a uniform medium and physical environment the major factors influencing culture yield are three in number: the time at which the observations are made, the size of the initial population and those limiting factors such as food supply and the conditions usually referred to as "crowding." In cultures of entozoic amoebae the food supply can be made the only limiting factor by supplying rice starch in quantities which will be completely utilized before the other effects become operative. The problem therefore becomes that of assessing the effect of these three factors with view to selecting that combination of conditions which will be the least subject to error.

In theory any cultivable strain of entozoic amoeba could be used for our purpose, but preliminary observations on strains of *Endamoeba histolytica* (3450 and NRS), *Endamoeba coli* (M-1 and 143), *Entamoeba invadens* (128) and *Entamoeba terrapinae* (127) indicated that the latter was most suitable for study. *E. histolytica* did not follow its life cycle in monobacterial associations and was critically sensitive to temperature variation. *E. coli* could not be induced to encyst and therefore could

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not be established with single strains of bacteria. It was, in addition, extremely sensitive to manipulation. *E. invadens* was similarly fragile. *E. terrapinae*, on the other hand, grew well over a wide range of temperatures, was easily established in a number of monobacterial associations, followed its complete life cycle in most such associations, and was relatively resistant to manipulation.

The usual practice in assessing variability is to use some arbitrary or empirically determined set of conditions as "normal" and evaluate deviations in terms of the "t" distribution (Snedecor 1946). Since in the present instance there is no such reasonable norm, a more rational approach is to use the factorial experiment (Fisher 1944, Snedecor 1946) and assess the variability by the analysis of variance and covariance. These are the general methods of which the various "t" tests are special cases. We have represented graphically the results of these analyses to render the "misbehavior" of the cultures more tangible.

MATERIALS AND METHODS.

The culture, (127), was originally obtained through the courtesy of Dr. C. W. Rees without designation other than its isolation from a turtle. Cytologically the trophic and cystic stages departed in no essential characteristic from the description given by Sanders and Cleveland (1930) for *Entamoeba terrapinae* spec. nov. By microisolation methods, essentially those of Rees (1942), a clone was established with an atypical strain of *Escherichia coli* isolated from the NRS strain of *E. histolytica*. In this association the amoebae followed their normal life cycle; the growth curve was somewhat extended; and the bacteria were not competitors for the rice starch. The culture was maintained at 30° C by transfer at four-day intervals in LSB medium with added rice starch. Bacterial purity was checked by repeated platings.

The medium used throughout was the liver-serum-broth (LSB) described by Griffin and McCarten (1949).

Rice starch was physically purified by the methods in the above citation.

Light component comprised the glutens and other low-density components of ground rice which appear as a brownish upper layer on centrifugation of trypsin-digested rice.

Yields were estimated by chamber counts in the manner previously described except that at the time of these studies elimination of excess starch by heating the formalized cultures had not been observed.

Experimental design. Computation of the analysis of covariance is much less burdensome if the ranges of the observations are divided into equal intervals so that Fisher's (1944) method of fitting orthogonal polynomials can be used in the manner described by Snedecor (1946). Pilot experiments indicated that the most fruitful ranges and intervals of the controlled variables would be: age, daily from 3–7 days; inocula by decimal increments from log inoculum 0–6; and rice starch in 5 mg units from 5–20 mg. The light component of digested rice was of unknown nutritional status so it was included as a fourth variable, the range being 0–25 percent of the total supply of rice in 5 percent increments. As the light component contains a considerable amount of fragmented starch grains an additional series was run with the cultures receiving only light component; the idea being that it

would give an estimate of the rice content if the glutens and other fractions should prove ineffective.

Preparation of inocula. The stock culture was subdivided repeatedly until a sufficient number of supposedly identical subcultures was at hand. At the time of use the supernate was removed and discarded, the sediments pooled, and the amoebae counted. If, at the time of counting, there was appreciable extracellular starch the pool was allowed to stand until such time as all of the starch grains had been ingested. On the basis of counts made at this time the pooled cultures were diluted with amoeba-free cultures of the bacteria to a density of 1,000,000 per ml. This suspension was decimally diluted using pure bacterial culture as the diluent and in sufficient volumes to permit inoculation of each of the requisite experimental cultures with 1.0 ml. of diluted suspension. The use of the amoeba-free cultures as diluent maintained a constant bacterial density.

Inoculation and incubation of cultures. With five ages, seven sizes of inoculum, four quantities of starch and seven concentrations of light component the entire study called for 980 individual cultures. It was not feasible to manipulate this number as a unit so each pool was used to inoculate cultures with all sizes of inoculum and all concentrations of light component but limited to a single starch quantity and from one to three ages. Eleven such pools were used over a three week period as shown in Table 1 where the entries are the dates on which the pools were used. The

TABLE 1.—*Pools Used for Inocula.*

Age (days)	Starch (mg)			
	5	10	15	20
3	7/13	7/26	8/10	8/5
4	7/15	7/26	8/10	8/5
5	7/7	7/19	7/27	8/5
6	7/17	7/26	7/27	8/1
7	7/9	7/19	7/27	8/1

cultures were incubated at 30° C for the required time whereupon they were killed and fixed by adding 10 percent of formalin by volume.

RESULTS

Since the analysis of variance showed that the light component contributed no significant part of the total variability, the light component concentrations serve as six replications of each age-inoculum-starch datum. The mean yields of these are given in Table 2. While the trend of yield with respect to log inoculum appears fairly uniform, even cursory inspection of the table shows gross irregularity in all other respects. When the data are subjected to the analysis of covariance the major trends are linear in nature except for the daily trends on log inoculum which are best fitted by a family of cubic equations.

Since the purpose of the study is to discover the sources of variation, it is the deviations from these trends which are our major concern. These are best illustrated as in Figures 1-3. The outer curves (A) are the 0.05 fiducial limits set by the variability arising from the main effect itself. The observed value at a given point should not lie outside these boundaries more than one time in twenty on the average. The inner curves (B) are the similar limits for all causes other than the main effect and the line (C) is the line of best fit for the observed data.

TABLE 2.
Mean Yields
Yields $\times 10^{-3}$.
Each original datum the average of 6 determinations.
Total number of cultures 840.

		Log Inoculum								Starch (mg.)			
		0	1	2	3	4	5	6	Mean	5	10	15	20
Age (days)	3	0	3	73	158	333	556	1302	346	299	240	585	259
	4	31	98	187	359	633	914	1526	536	351	512	920	360
	5	18	33	137	340	752	840	987	444	305	261	688	523
	6	143	375	720	1069	1107	1033	868	759	403	791	682	1162
	7	149	426	539	966	1015	766	407	610	281	503	711	943
Mean		68	187	331	579	690	947	1273	539				
Starch (mg.)	5	91	132	278	364	407	406	614		328			
	10	81	81	188	479	789	818	796			462		
	15	24	98	281	925	1185	1117	1388				717	
	20	77	436	577	547	690	947	1273					649

If the total variability is uniformly distributed over the entire experiment the separation of curves (A) and (B) should not be statistically significant. This is true only of the effect of size of inoculum (Fig. 2), the variability associated with age alone (Fig. 1) and quantity of starch alone (Fig. 3) being far in excess of reasonable random occurrence. Here, then are the major sources of variation, but it should be noted that this variability is not necessarily a function of lack of precision in determining the age at which the counts were made or measuring the amount of starch. The analysis of covariance does not specify the nature of the error, only the effect with which it is associated.

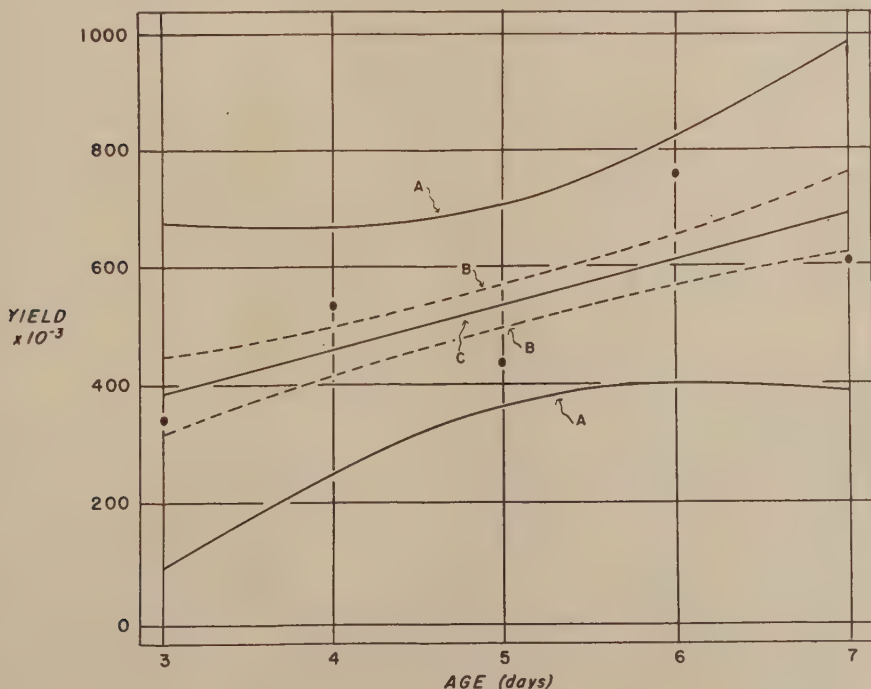


FIG. 1. Covariance of yield with age of culture.

Were there no interaction of main effects it would be impossible to select a combination which would minimize error. It is, however, a logical necessity that yield at a given age will depend on the size of inoculum and amount of starch available. The analysis of covariance showed that the major trend of interaction was the tendency for the daily trends to vary as the third power of log inoculum. The starch-age-inoculum interrelationship is best derived from other considerations. It is essentially linear.

The equations of best fit for each size of inoculum are illustrated in Figure 4. It is apparent that the most rapid growth was from an inoculum about 1,000, the expected value is about 1,700 ($\log I = 3.23$), and that an inoculum somewhat in

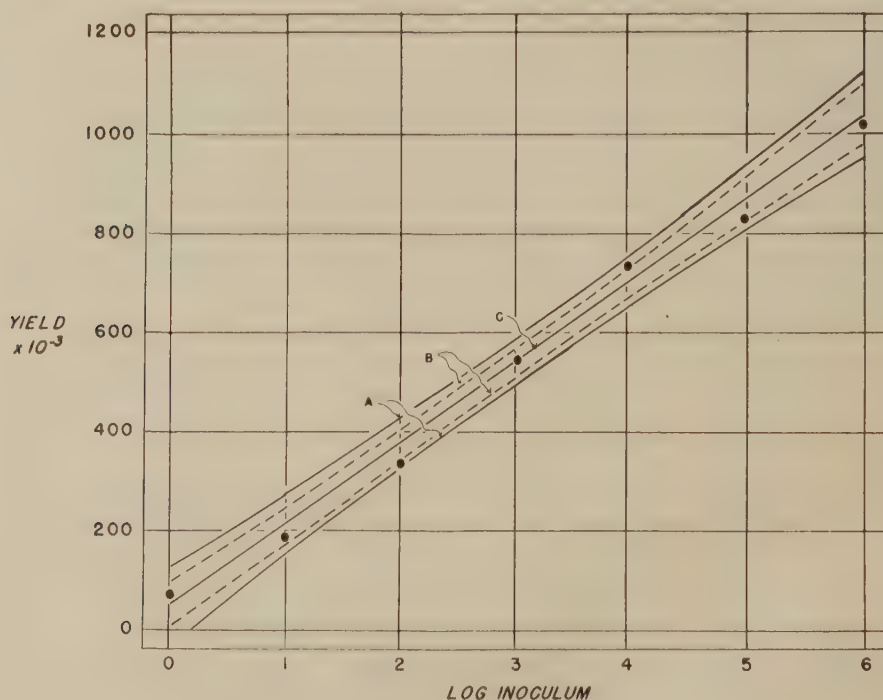


FIG. 2. Covariance of yield with size of inoculum.

excess of 100,000 should give a static population. The latter is computed as about 170,000 ($\log I = 5.23$). The yields will be least subject to error if yields are determined on the sixth day and inocula in excess of 10,000 are used.

The similar treatment of the daily yields results in the family of equations illustrated in Figure 5. It is apparent that the linear behavior of log inoculum as a main effect is due to the fortuitous choice of the range of ages and quantities of starch. Since the sixth day was the most suitable age the inoculum should be such as to give a maximum at that time or about 50,000 ($\log I = 4.75$).

The negative curvature of the curves after the fifth day can be shown to be the result of exhaustion of food. While it is possible to develop an equation for the interrelationship of age-inoculum-starch by eliminating yield from the multiple

correlation, similar, and possibly more convincing, results can be obtained by direct observation. As the counts were made the presence or absence of starch was recorded. From these records the inoculum required to exhaust the starch in a given time was computed. Thus in seven days all cultures with an inoculum corresponding to $\log I = 3.2$ or greater would utilize all the starch. The greater the inoculum, of course, the greater the rapidity of use. This characteristic is indicated by the discontinuous parts of the curves of Figure 5, except that an inoculum of $\log I = 7$ would theoretically be needed to exhaust the starch in 3 days. The negative curvature of these regressions is thus due to the progressive diminution of yield in the six and seven day cultures, as seen in Figure 4, and is directly related to the food source.

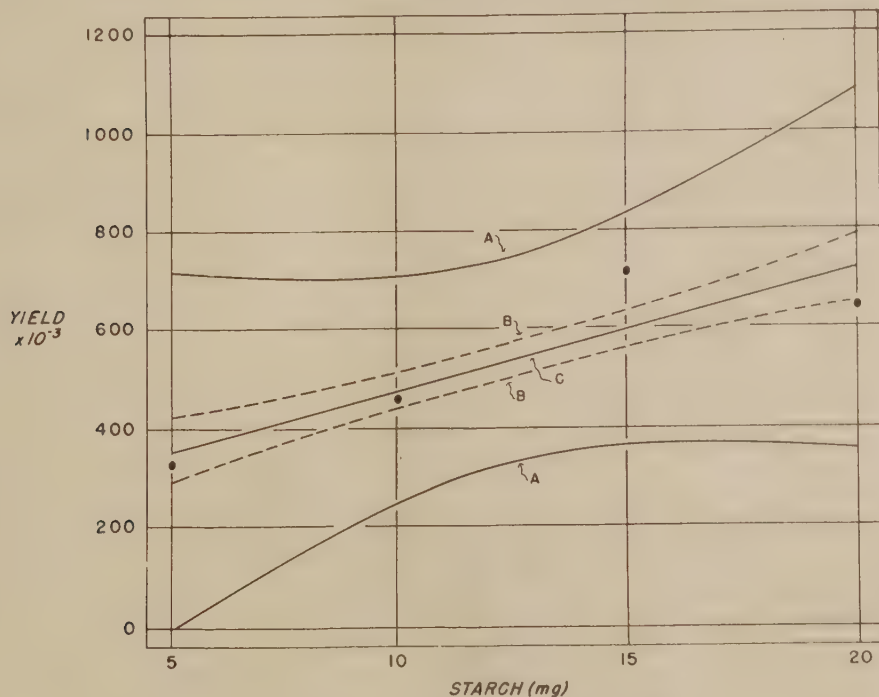


FIG. 3. Covariance of yield with quantity of starch.

We may then compute that a minimum of 20 mg. of starch is required to maintain the cultures for the desired incubation period. We believe that any excess should be slight as such excesses may be inhibitory under some circumstances.

While the physically pure rice starch used in these experiments can not be considered a chemical entity it does have the advantage of having had a variable amount of inert material eliminated. Some idea of the relative amounts of the inactive components may be gained as follows. In the LSB medium *E. terrapinae* does not survive without starch. Since the addition of light component was without effect we may reasonably assume that growth in 100 percent light component is due to the fragmented rice particles inevitably mixed with the glutens. The mean yield from 140 cultures receiving only light component was 325×10^3 as compared with

539×10^3 for rice starch alone. Light component therefore is about 60 percent starch and 40 percent inert material. Since trypsin-digested starch contains about 20 percent of light component such digests are about eight percent inert material. In similar experiments using whole ground rice, the mean yield for 140 cultures was 343×10^3 or 63 percent of that obtained with purified rice. Inasmuch as the weight loss of whole rice on tryptic digestion is about 40 percent, the available evidence does not indicate the presence of appreciable growth stimulants in polished rice other than the starch.

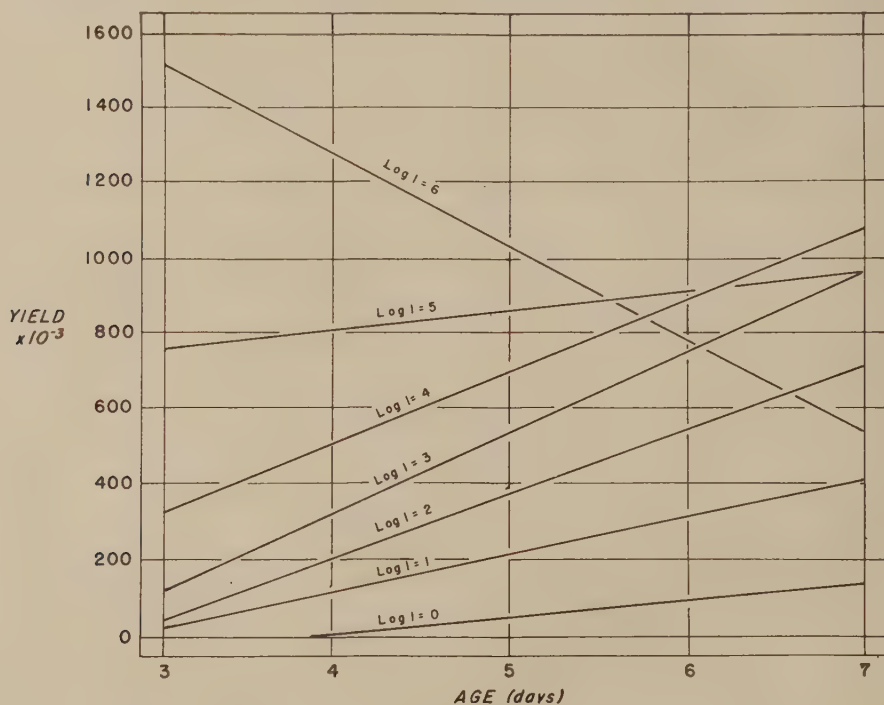


FIG. 4. Regressions of yield on age of culture by sizes of inoculum.

DISCUSSION

In these experiments the results point to uncontrolled variation associated in some way with age of culture and quantity of starch. The statistically minded will immediately note that this non-random distribution of the variation is due to the fact that the effect of the inoculating pools is concentrated in the ages and starch quantities but uniformly distributed over the sizes of inoculum. Our assumption of constancy in such cultures pooled at different times was therefore erroneous. There are two sources of such inconstancy which are subject to experimental evaluation. First, the cultures were not of precisely the same age when pooled. It will be recalled that the pools were allowed to stand until there was no excess starch. This may have resulted in several hours' difference in age of inoculum. Furthermore the pool cultures were not quantitatively inoculated with the result that some cultures

had doubtless been without starch for some time whereas others still had an excess of starch and were vigorously growing. Secondly there is evidence of periodicity in reproductive potential. Craig (1948) calls attention to the commonly experienced cyclical variation in growth of cultures of *Endamoeba histolytica* and serial experiments carried out in this laboratory over a period of a year lead us to the conclusion that this rhythmic behavior is a part of the life pattern of the organism. Thus if a culture is on the upswing, yields from successive inocula will increase under otherwise identical conditions, a phenomenon which will be reversed as the growth potential passes its peak and proceeds down grade.

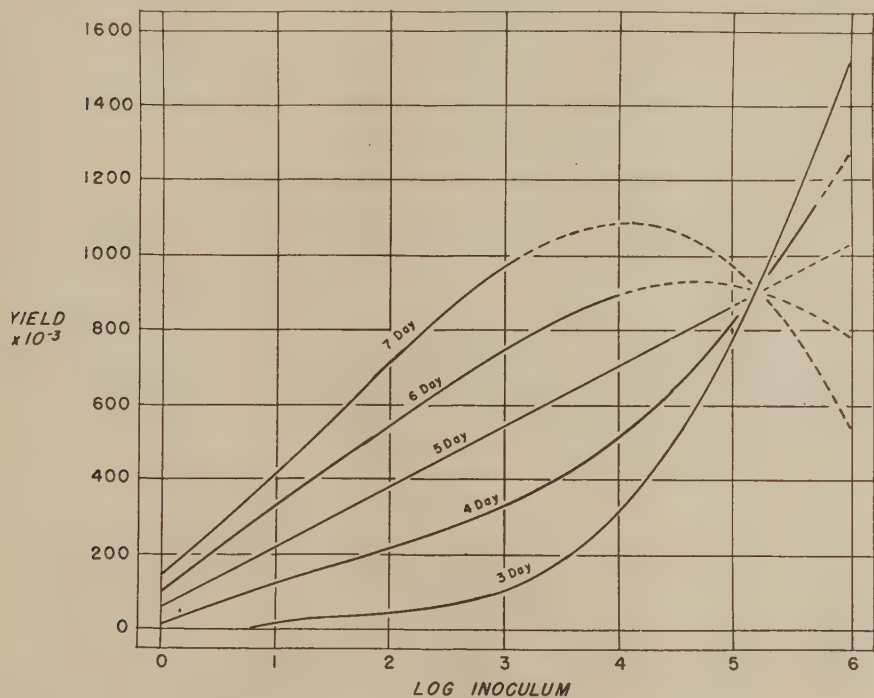


FIG. 5. Regressions of yield on size of inoculum by ages of culture.

Our results were obtained with a single strain of amoeba under the highly restricted conditions attending a monobacterial association. We would hesitate to assert that these findings would apply in detail to other populations. There is, however, ample reason to believe that the major differences will be of detail rather than principle. At least as a basis for further studies the indications are that the most consistent results will be obtained when inocula, time of observation and starch supply are balanced to give maximum yields. The specific values for a particular culture may be expected to vary and must be uniquely determined.

SUMMARY

1) Application of the factorial design to *in vitro* experimentation with entozoic amoebae permits segregation of the sources of variation.

2) The major sources of variation were associated with age of culture and quantity of starch.

3) It is suggested that this variation is associated with inconsistent behavior of the cultures used for inoculation.

4) Error may be minimized by selection of that combination of age, inoculum and quantity of starch which gives yields near the simultaneous values of interacting effects. For the experiments reported these were respectively: six days, 50,000 and 20 mg.

5) It is suggested that the variability of inocula may be associated with exhaustion of food and periodic variation of growth potential.

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SOME SOURCES OF VARIABILITY IN CULTURES OF ENTOZOIC AMOEBAE. II. THE EFFECT OF AGE OF INOCULUM*

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In the preceding report Griffin and McCarten (1950) showed that most of the excess variability in cultures of an entozoic amoeba was inherent in the pooled cultures used for inoculation. They suggested that this might be due to variation in the age of the inoculating cultures and to periodic variation on the growth potential of the organisms. The following study is an attempt to resolve the factors involved in the first of these.

MATERIALS AND METHODS

The culture. Subsequent to the work previously reported our reptilian amoeba strains were lost by exposure to sulphur dioxide. Since our (127) strain of *Entamoeba terrapinae* could no longer be obtained elsewhere we isolated a number of amoebae from common laboratory turtles, presumably of the genus *Pseudemys*. One of these (165) was morphologically identical with *E. terrapinae* but differed from strain (127) in some respects. No monobacterial association could be established which permitted completion of the life cycle and with the atypical *Escherichia coli* previously used, growth was so poor as to cause us to discard the culture after a few weeks. Since Clinkston (1948) was able to demonstrate the constancy of a mixed bacterial flora in the quantitative sense we felt justified in using strain (165) in association with the stabilized flora from the original isolation.

The medium was the liver-serum-broth previously described, supplemented with 25 mg of purified rice starch.

The experiment was designed to assay the effect of age of inoculum correlated with age of culture. The 25 mg. quantity of starch placed a sharp limitation on growth at about 48 hrs. so that the results could be tested for conformance to the hypothesis of exponential growth and death of cultures. Datum points for both age of inoculum and age of culture were by 12 hr. increments up to 48 hrs. and by 24 hr. increments from 48 hrs. to 144 hrs.

As an added contribution to uniformity, a clone was established from the original culture. From this substrain, cultures were inoculated with 50,000 amoebae at 48 hr. intervals until an adequate number of subcultures was available to provide inoculum for the entire experiment.

At the indicated times the requisite number of cultures was withdrawn from stock, pooled, and the density adjusted to 50,000 per ml. by the addition of amoeba-free supernate. Each of 40 tubes was inoculated with 1.0 ml. of amoeba suspension, providing five replicates for each of eight ages of culture. All cultures were incubated at 30° C. for the required time at which point the yields were determined by the methods described by Griffin and McCarten (1949).

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RESULTS

The mean yields of the five replicates for each age of inoculum and age of culture, together with their coefficients of variation (ratio of standard deviation to mean as percent), are given in Table 1. There is a notable tendency for the lower

TABLE 1.—Mean Yields ($\times 10^{-3}$) and Coefficients of Variation of Replicate Cultures

Age of Culture (hrs.)	Age of Inoculum (hrs.)									Mean
		12	24	36	48	72	96	120	144	
12	Mean	73	81	55	99	102	151	111	115	98
	C	14.8	10.7	40.2	17.6	5.9	3.7	14.7	22.5	
24	Mean	171	352	334	510	704	312	266	267	364
	C	10.4	6.1	10.2	8.4	8.0	3.7	11.1	5.4	
36	Mean	867	739	900	1011	1678	798	561	420	874
	C	7.9	10.7	10.1	8.2	4.0	20.9	8.8	20.3	
48	Mean	889	2682	2297	2671	1740	1336	1297	925	1730
	C	34.9	6.3	10.7	15.5	11.2	12.2	11.6	4.8	
72	Mean	1848	1306	1859	1754	1426	1479	748	1126	1443
	C	36.8	21.3	15.6	17.6	35.2	12.6	12.9	10.7	
96	Mean	1851	1281	1256	1422	1676	1297	563	718	1258
	C	26.9	19.8	10.7	9.8	40.9	16.1	13.8	24.8	
120	Mean	1510	996	1141	1417	1285	830	599	405	1022
	C	31.4	15.2	5.7	13.8	23.5	30.6	40.2	122.1	
144	Mean	561	720	1039	1283	1204	838	577	767	874
	C	3.8	17.0	37.5	15.3	9.4	8.0	26.3	31.1	
Mean		972	1020	1110	1271	1227	880	590	593	

coefficients of variation to be associated with cultures less than 48 hrs. of age. The extent of this phenomenon will be considered later.

The analysis of variance showed an unexpected degree of interaction between ages of inoculum and culture. The general trend of this interaction can be seen in Table 1 where the maximum yields tend to occur later with younger inoculums, but for our purposes there is no need to subject it to further analysis.

On the basis of the hypothesis of exponential trends with respect to age of culture (Richards 1941), the relationship between yield and age of culture can be expressed as:

$$Y = Y_0 e^{kt}$$

where: Y = yield

Y_0 = inoculum, i.e., yield at zero time.

e = the base of natural logarithms

k = a proportionality constant

t = time

For purposes of analysis this equation can be transformed by taking logarithms of both sides to give the linear type:

$$\log Y = \log Y_0 + kt$$

and used in this form for adjusting the observed mean values. Since the experiment was designed to give a critical point at 48 hrs. the results are separated into two portions, a period of increase from 0–2 days and a period of decrease from 2–6 days. The curves of best fit for age of culture are given in Figure 1. Agreement with hypothesis is excellent, the standard deviation from regression being but 0.097 for the first period and 0.013 for the second. The computed values of k are 1.90 and

-0.17 respectively, giving a generation time of 8.8 hrs. and a "death" time of 4.1 days. These values are consistent with empirical observations made in another connection. The computed value of Y_0 for the growth period is 45,000 which agrees well with the stated inoculum. The similar value for the period of decline is a measure of the maximum supportable population, 2,400,000, which is sufficiently close to the average yield of 2,500,000 obtained when similar cultures are supplied with adequate starch and allowed to reach their greatest development.

It is interesting to note that the same type of relationship holds for yield and age of inoculum, except that the constants have different connotations. The adjusted means are illustrated in Figure 2. The computed k 's indicate that in a growing

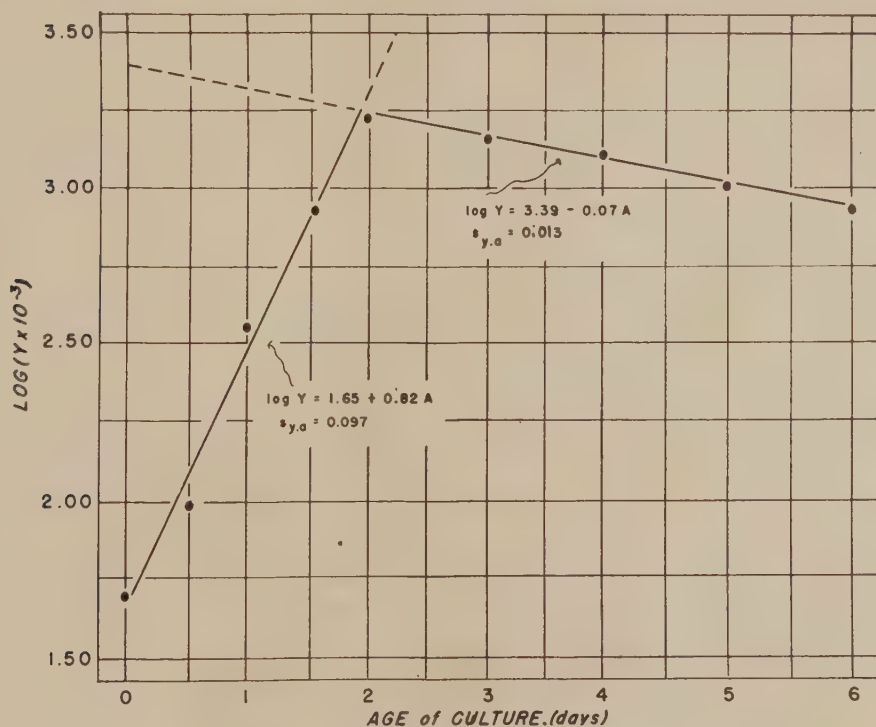


FIG. 1. Regression of Log Yield on Age of Cultures.

culture the "growth potential" of the organisms increases at the rate of about 26 percent per day ($k = 0.18$) whereas in a dying culture this capacity for reproduction decreases at about 33 percent per day ($k = -0.23$). Extrapolation of the period of increase to zero time gives a value for Y_0 of 870,000. This is the implied yield from cultures freshly transferred and immediately retransferred. It seems to us that this is probably due to the shock of transfer to the new environment from which the organisms gradually recover. Similar extrapolation of the period of decrease indicates a maximum potential yield of 2,100,000 which is not significantly different from that computed from Figure 1.

Gratifying though these results are, there is still a great deal of residual varia-

bility in the experiment. The excellent fit of the mean values to expectation is due, in part, to the fact that each is the average of forty determinations. This number of replications is scarcely feasible in ordinary experimentation. It will be recalled that Table 1 showed a concentration of high coefficients of variation in cultures more than 48 hrs. old, i.e., in the "death phase." To assess the distribution of such variability it is necessary to return to the unconverted counts as made in the chamber, since the multiplying factors used to compute the yield per culture affect the test for homogeneity of replicates. The use of chi-square as in Table 2 to test this

TABLE 2.—*Chi-square' Test for Homogeneity of Replicate Cultures*

Age of Culture (hrs.)	Age of Inoculum (hrs.)							
	12	24	36	48	72	96	120	144
12	0.605	0.390	3.470	1.210	0.140	0.079	0.950	2.112
24	0.738	0.515	1.396	1.438	1.790	0.169	1.519	0.310
36	3.380	3.380	3.645	2.713	1.098	13.940**	1.721	6.930
48	43.320***	4.260	10.510*	25.810***	8.769	8.040	7.068	0.858
72	99.980***	23.660***	18.150**	21.620***	70.600***	9.360	4.992	5.130
96	53.070***	20.010***	5.740	5.490	111.980***	13.530**	4.329	17.632**
120	62.070***	9.180	1.500	10.740*	28.340***	31.024***	38.704***	241.570***
144	0.339	8.280	58.590***	11.960*	4.230	2.143	15.991**	29.543***

¹ d.f. = 4

* Probability 0.05 - 0.01

** " 0.01 - 0.001

*** " less than 0.001

internal homogeneity places a severe restriction on the permissible variation. In effect it is a test of whether replicate cultures vary more than is inherent in the counting method. Even so it is notable that significant departures from homogeneity are the exception prior to 48 hrs. of culture age and the rule thereafter, whereas the distribution with respect to age of inoculum is uniform. The total chi-square for the first 48 hrs. of growth is 162.273 which has a probability of random occurrence of 4.0 percent. Since some variability among the cultures is inevitable it is surprising that the results are so close to the 5.0 percent level of significance. The total chi-square for the period after 48 hrs. is 1,039.477 with a random probability far less than 0.001 percent.

DISCUSSION

As a result of these observations it is possible to specify the conditions under which the results of a given experiment will be subject to statistical error little greater than that involved in the counting technique. In addition to the obvious specifications of uniformity of media and physical environment these are: (1) a constant optimum inoculum, (2) sufficient purified starch to permit continued growth over the period of the observations, (3) determination of yield at a constant time within this period of growth. If maximum yields are desired a fourth specification must be added, that inocula must be taken at the point of maximum growth.

These specifications will permit the evaluation of differences only slightly greater than the limitations of enumeration which can, of course, be reduced to any desired level by replication of counts.

The methods used in this search for variation are also applicable to the determination of the characteristics of modified media. Instead of doing numerous counts over a considerable period of time one can, with a minimum of preliminary experimentation, arrange conditions to introduce a sharp flexion between growth and death of the cultures. With some sacrifice of precision the properties of the

medium can be estimated with two determinations of yield during the growth period and two during the death phase. Thus for strain (165) with its native bacterial flora the LSB medium will give an average maximum yield of about 2,400,000 per 10 ml. culture and this maximum will be reached on the average at 2.1 days. To achieve this maximum yield the inoculum must be from a culture close to this age. One can similarly estimate the properties of the medium with respect to its effect on reproductivity by similar experiments varying the age of the inoculum.

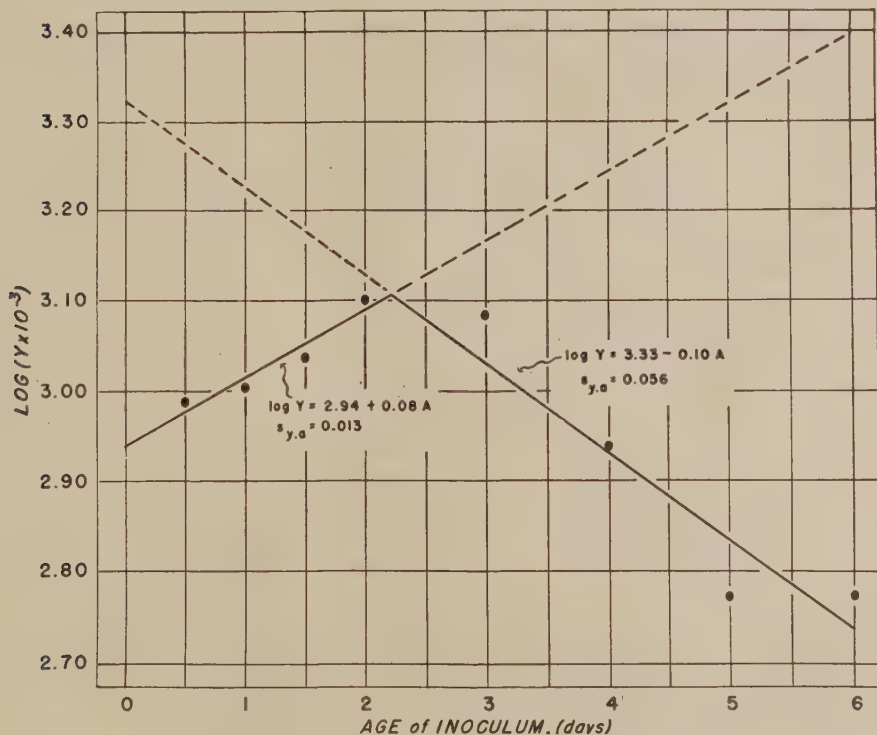


FIG. 2. Regressions of Log Yield on Age of Inoculum.

We feel that it is necessary to stress the fact that such uniformity of results can be expected only in a given experiment. A subsequent report will substantiate the suggestion by Griffin and McCarten (1950) that there is a periodicity of growth potential in amoebic cultures which is not correlated with the age of inoculum effect reported in this paper. Thus unless serial experiments are initiated with inocula at the same point on this curve of potential the results will differ considerably and precision will be lost. This specification may prove impracticable, however, due to the length of the cycle and a more rational approach would be serial repetition of the experiments with the variation associated with such repetitions being segregated by the analysis of variance.

Finally we strongly urge the use of the factorial design of experiment with the analysis of variance serving as a statistical control throughout the progress of any

line of investigation rather than a terminal procedure. By this means we have frequently been deterred from continuing series of experiments which would have had meaningless results on completion.

SUMMARY

1) The statistical error in a given experiment with cultures of *E. terrapinae* can be reduced to little more than that inherent in the counting technique if, in addition to uniform media and physical environment: (a) a single pool is used to inoculate all cultures, (b) a constant and optimum inoculum is used, and (c) yields are determined within the period of logarithmic growth.

2) The properties of the growth and death curves permit evaluation of the maximum potentialities of a given medium with respect to maximum yield, rate of growth and rate of survival.

3) It is stressed that the above specifications apply only to single experiments and not to the comparison of chronological repetitions.

4) Attention is called to the desirability of the factorial design and statistical control of the progress of experiments.

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SOME SOURCES OF VARIABILITY IN CULTURES OF ENTOZOIC AMOEBAE. III. VARIATION IN VITALITY*

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In the first paper of this series (Griffin and McCarten, 1950) we suggested that a major part of the variability in cultures of *Entamoeba terrapinae* was due to inconstant vitality in the cultures used as inoculums. In those experiments eleven pools were used over a three week period. Subsequently Griffin and Michini (1950) showed that statistically consistent results could be obtained within a given experiment, but this does not permit direct comparison of experiments done in sequence unless it can be shown that the controls do not vary significantly.

Periodic variation in protozoan cultures has been recognized for decades (Richards, 1941), but we have been unable to find any report of systematic observations of cultures of entozoic amoebae based on quantitative methods. Certainly if such changes in vitality are large and occur within short periods of time they must be taken into account in the design of experiments. This is a report of fourteen months' continual observation of the vitality of our (165) strain of *Entamoeba terrapinae*.

EXPERIMENTS

Culture (165) was isolated from a turtle received from a laboratory supply house on 10-25-47 and established in our LSB medium. It was transferred in the usual manner at 3-4 day intervals, being maintained at a constant temperature of 30° C. On 5-20-48, after seven months of satisfactory subcultures, the serial experiments were begun. At the time of transfer the cultures were mixed as thoroughly as possible without resorting to violent agitation and 0.1 ml. transferred to 10 ml. of fresh medium containing 25 mg. of purified rice starch. There were five series of duplicate cultures, one for each transfer interval from 2-6 days inclusive. Successive transfers were made in the same manner. The choice of constant volume rather than constant numbers for inoculums was dictated by two considerations: (1) pressure of other duties often precluded taking the time necessary to make the counts when transfers were due and (2) we felt that with an appropriate choice of volume the variations in vitality would be accentuated since increasing vitality would give increasing inoculums and vice versa which would be reflected in the yields. At the time of each transfer the remainder of the culture was formalized and set aside for counting as opportunity permitted.

With an inoculum of 0.1 ml. the two-day series were lost after 14-25 transfers. The inoculum for this series was therefore increased to 0.2 ml. and a new series started on the 86th day of the experiment. The three-day series survived for 126 transfers, but the four-, five- and six-day series were lost after 25-30 transfers. It might have been possible to reestablish the last three on the basis of some other in-

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oculum, but the experiment had been under way for more than four months and we felt that adequate information could be obtained from the remaining two series.

The technique of these experiments violates deliberately many of our specifications for uniformity in amoebic cultures. The resulting variation among transfers makes necessary the use of moving averages with a span of 15 transfers to bring the trends into definite focus. These trends (Figure 1) for the two- and three-day

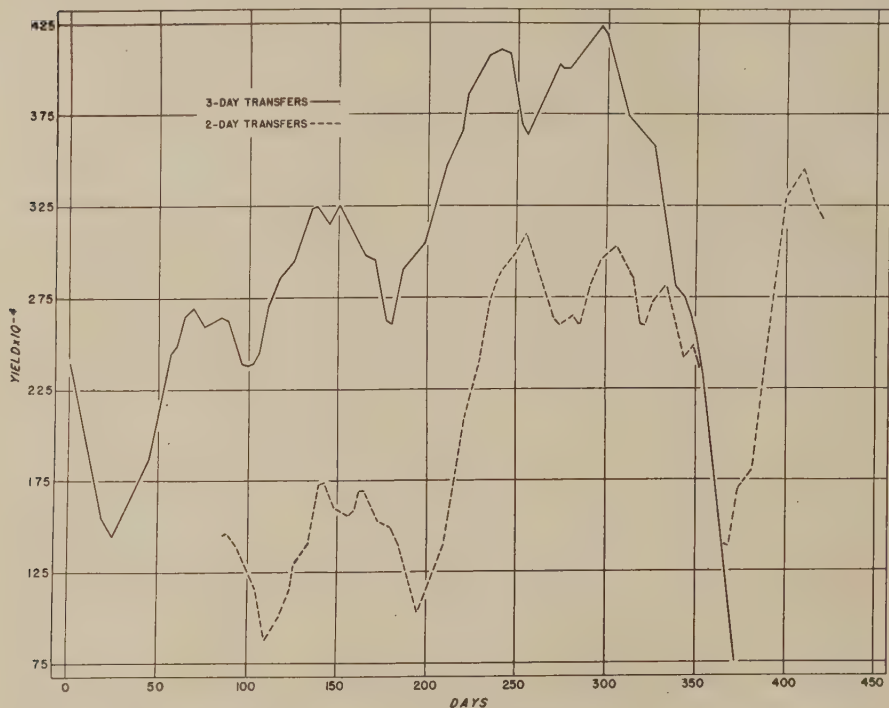


FIG. 1. Mean yields of cultures of *E. terrapinae* transferred at two- and three-day intervals.

series have been drafted precisely in the neighborhood of critical points but, for the sake of clarity, have been smoothed within a range of plus or minus the standard error of counting for intermediate values. The time scale is for the entire experiment, so the two-day series starts on day 86.

The general aspects of the two trends are remarkably similar up to 350 days although there is some tendency for the two-day series to lag. On the 354th day the two trends become so nearly identical that they can not be drafted as separate curves on the scale of the chart. The three-day series failed to recover from this depression phase and was lost on the 375th day.

Ignoring such dubious notchings as occur in the three-day trend at 75 and 145 days, there is notably uniform periodicity in both trends. There is a period of approximately 90 days in which vitality increases for 50–60 days and decreases for 30–40 days. Two of these periods seem to combine to give a cycle about 180 days in length as seen in the span 195–364 days in the two-day trend and 160–365 days in

the three-day trend. Finally the complete cycle for the three-day trend has taken about a year as is also true of the two-day trend if we assume that it would have paralleled the other curve prior to 86 days. This annual cycle is still in evidence although the continuous experiment was terminated on 7-13-48. The two-day series has been continued as one of our stock cultures and serial counts during November 1949 and January 1950 show a minimum about 11-10-49 and a maximum about 1-26-50. These dates are just about a year after the corresponding points at 194 and 256 days on the two-day curve.

If one connects the points of maximum yield it appears that the whole experiment is part of a larger trend. The highest average yield in the two-day series was 320×10^4 at 420 days. About six months later the corresponding maximum was 393×10^4 . The period of this major cycle can not yet be predicted. The culture had just passed the above peak at the time of writing when the strain had been under cultivation for more than thirty-nine months. The cycle is therefore at least six years in length if the phenomenon of proportionality persists.

There have been many observations presumptively relating encystment of entozoic amoebae to growth rate, a phenomenon which has been the basis for many of the techniques used to induce encystment. The present study gives excellent confirmation of this relationship between encystment and vitality. In Figure 2 are given the trends for relative cyst production correlated with total yield. These are for the three-day series since few cysts are formed in two days even in rapidly growing cultures. The absolute correlation between encystment and vitality is obvious.

DISCUSSION

Our intention at the start of these experiments was to determine the maintenance regimen least subject to variation, the number of serial repetitions of experiments necessary to give a satisfactory measure of this type of variation, and the best spacing of such repetitions. As the data accumulated it became apparent that we were dealing with a basic biological phenomenon not, within our ability to foresee, subject to external control. We therefore decided to extend our observations beyond our original 30-60 day estimate and attempt to describe the periodicity as completely as possible.

It is apparent from the data presented that periodicity is a function of the total age of the culture rather than the number of frequency of transfers. Our limited experience with the longer transfer intervals indicated that those series were also following cycles parallel to those described in detail. Because of the potential loss under other regimens we have adopted the two-day transfer interval for the (165) strain of *Entamoeba terrapinae*, but this would not necessarily be the most efficient procedure with other strains and species.

In judging the effect of experimental conditions it is absolutely obligatory to incorporate this "vitality effect" into the design. This is particularly true if two series happen to be out of phase. For example, were we to compare the effect of three-day transfer with two-day over the 15-day period beginning with 180 days we would conclude that the three-day regimen was favorable and the two-day unfavorable. Exactly the opposite conclusions would have been drawn had we started our observations on the 240th day and continued for a similar time. Fortunately these effects can be segregated by serializing experiments with each factor being carried forward a

sufficient number of times and using a constant-number inoculum throughout. The exact number of repetitions will depend on the state of the culture and can not be predicted in advance. It has been our practice to maintain a state of statistical control and continue serial replications until the effects of "transfers" and the transfer interactions assume clear cut characteristics. At times of relative stability three or four repetitions may be sufficient, at other times we have had to carry the experiment through nine or ten transfers before we could get an adequate assessment of the desired effects.

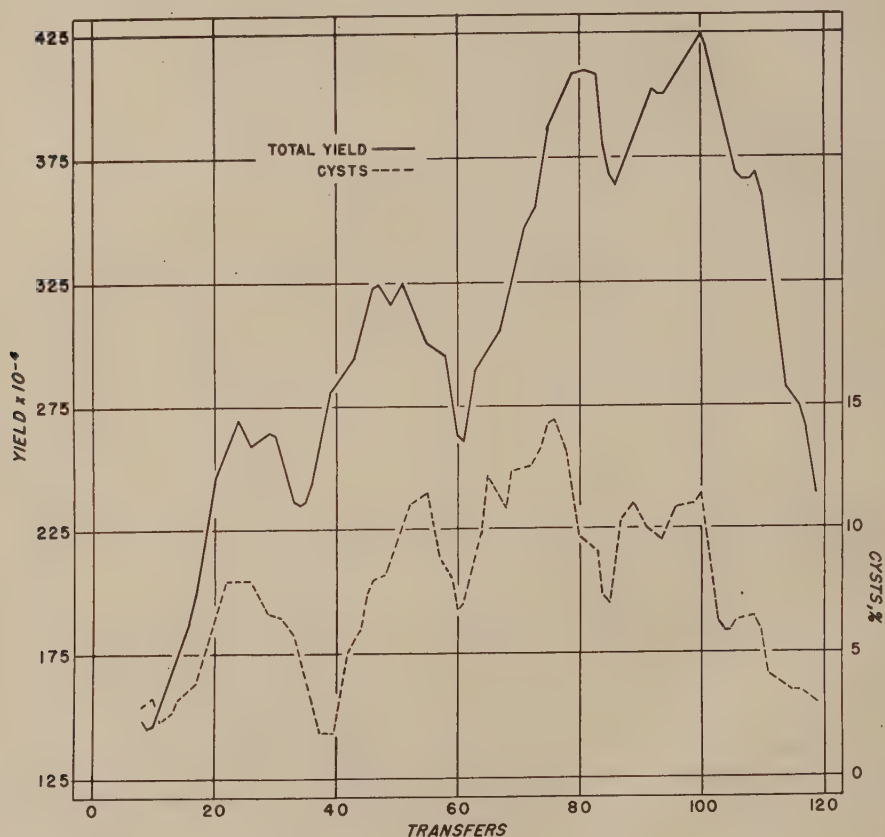


FIG. 2. Relative yields of cysts in serial three-day cultures of *E. terrapinae*.

We are unable to offer any hypothesis which seems adequate to us as to the cause of the observed periodicity. Cyclic changes in vitality of protozoan cultures has been variously ascribed to inadequacy of the medium, endomixis, and environmental effects (cf. Richards, 1941). The first of these may result in extremely long cycles, as shown by Richards and Dawson (1927), but the effect is one of gradual loss of vitality whereas we have observed a gradual increase and can only postulate the ultimate decrease. The sudden loss of the three-day series may be due to inadequate inoculum, but in any event is not at all comparable with the isolation cultures used in pedigree studies. Endomixis in amoebae is of dubious status (cf. Turner, 1941)

and even if intracystic reorganization of nuclear material were to occur, as suggested by Mackinnon (1914), the period of increased vigor should follow a period of increased encystment instead of being synchronous with or preceding it. The physical environment of the cultures has been as constant as our technique will permit. There is no correlation between periodicity and the use of new batches of medium or starch. The cultures have been maintained at a constant 30° C. and in the dark except for the brief intervals during transfers.

The seasonal nature of the cycles is intriguing, but would require a rather remarkable "racial memory". The organism studied is normally a parasite of a cold-blooded animal and might, in nature, have had an impressed seasonal activity. It was, however, placed in an unnatural environment of continuous high summer temperature and relieved of possible seasonal starvation, yet the annual variation appears to have continued more than two years.

The associated bacteria have a profound effect on protozoan cultures, but it is most unlikely that changes in the bacterial flora are responsible for the periodicity. Since completing the above study we have had opportunity to observe the amoebae in a variety of floras and periodicity has always been present. Furthermore it strains our credulity to assume that we conveniently contaminated all our cultures at regular 90-day intervals with cooperative contaminants that uniformly disappeared in 30–40 days.

However, since our current studies lie in other directions we must be content merely to record the occurrence of cycles without constructive suggestions as to their cause.

SUMMARY

- 1) Cultures of *Endamoebae terrapinae* showed cyclic alterations of vitality with periods of approximately 90, 180 and 360 days. There was evidence of still longer periods.
- 2) The periodicity was associated with total age of culture rather than frequency of transfer.
- 3) Encystment was positively correlated with vitality.

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BLOOD PROTOZOA OF BIRDS AT COLUMBIA, SOUTH CAROLINA¹

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As part of a study on the susceptibility of various mosquitoes to avian malaria, the incidence of blood protozoa in some common wild birds was investigated.

Birds were trapped on the grounds of the State Hospital at Columbia, S. C., from February 1947 to August 1949. Of the 737 birds caught, 479 were taken during the months of June, July, and August, 1948 and 1949.

Usually, a bird was examined only one time. Blood smears were made from the superficial leg or wing veins. The blood smears were stained with Giemsa and examined under an oil immersion lens for at least ten minutes each. Parasites which could not be positively identified because of their scarcity or for other reasons were recorded as unidentified.

Table 1 shows the data relating to the incidence of blood protozoa in the various birds. Of the 737 birds, 203, or 27.5 percent, had some type of blood protozoa; 129, or 17.5 percent, had plasmodia. Forty-two birds had multiple infections, of which 38 were double, three triple and one quadruple. There were thus 250 blood protozoal infections in the 203 parasitized birds. There were 138 plasmodial infections in the 129 birds with malaria.

The cardinal showed the highest incidence of *Plasmodium*, eight of the nine birds being infected. The grackle showed an incidence of 25.6 percent and the English sparrow 13.3 percent. The grackles showed a variety of infections, 43.5 percent having some blood protozoa. Of the 131 infected grackles, 33 had multiple infections including one individual with parasites belonging to four different genera, viz., *Plasmodium* sp., *Haemoproteus*, *Leucocytozoon*, and *Trypanosoma*.

P. relictum was by far the most prevalent *Plasmodium*, accounting for 67.0 percent of those identified definitely as to species.

With the lists of Herman (1944) and Hart (1949) as a basis, seven new host records for *Plasmodium* were found. These are indicated by asterisks in Table 1.

The incidence of plasmodial parasites found during the summer months of June, July, and August was compared to that for the remainder of the year in the birds most frequently examined. The grackles showed 26.1 percent infected of 215 examined in the summer as compared to 24.4 percent of 86 examined during the remainder of the year. These rates do not differ significantly.

Of 178 English sparrows examined during the summer, 9.6 percent had plasmodia as compared to 27.7 percent of 47 of these birds examined during the rest of

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TABLE 1.—Distribution of blood protozoa in birds caught at Columbia, South Carolina

[illegible]

* New host record.

* New host record.
** As there is so little difference between the Florida and purple grackles, they are considered as the same hosts in this report. Should the Florida grackle be considered as a distinct host, then all of the infected grackles shown in Table 1 would be new host records.

the year. The latter rate is significantly higher ($p = \text{approx. } .001$). This is similar to the findings of Hart (1949) who at Manning, S. C., found a winter rate in English sparrows twice that of the summer rate. Recently, Micks (1949) found English sparrows with the highest incidence of plasmodia during the warmer months but most of his birds came from an area other than South Carolina.

SUMMARY AND CONCLUSIONS

Of 737 birds caught at Columbia, S. C., 27.5 percent showed some blood protozoa; 17.5 percent showed *Plasmodium*. *Plasmodium relictum* accounted for 67.0 percent of the plasmodia identified. In English sparrows, the plasmodia appeared to be less prevalent during the summer than during the remainder of the year.

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TRICHOMONAS MICROTI, N. SP. (PROTOZOA, MASTIGOPHORA)

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INTRODUCTION

Until 1911, apparently only one species of *Trichomonas* had been recognized from the caecum of rats and mice. This was sometimes considered to be *T. muris* (*Mono-cerconomas muris*, Grassi, 1879) or *T. intestinalis* (Leuckart, 1879). While Wenyon (1907) employed only Leuckart's name for trichomonads from mice, he recorded a great range in length from 3 to 20 microns. Galli-Valerio (1907) and Hartmann (Kisskalt und Hartmann, 1910) gave the name *T. muris* to what appear to be the larger forms. In 1911 Alexeieff briefly described *T. parva* as a new small species from the rat, but the description was too meager to permit clear identification. In 1921 Wenrich noted a smaller species which might be *T. parva* Alex. In 1924 the same author briefly described the following: *T. muris* Hartmann, *T. parva* Alex., *T. minuta*, n. sp., *Pentatrachomonas* sp., possibly identical with the *Pentatrachomonas* of man, and *Pentatrachomonastix muris*, n. gen., n. sp. In 1930, Wenrich offered a diagnostic key and illustrations for the intestinal flagellates of rats and placed *Pentatrachomonastix muris* in the genus *Hexamastix*.

In 1931, the senior author attempted to resolve some of the taxonomic uncertainties by sending to Alexeieff a drawing of each of the trichomonads mentioned above asking him to identify *T. parva* and return the drawings. Instead, Alexeieff kept these drawings and sent back two of his own, one of *T. muris* from the mouse and the other of *T. parva* from the rat. While the drawing of *T. parva* showed only three anterior flagella, the other characters were those of *T. hominis* and did not apply to the species tentatively identified as *T. parva* by Wenrich (1924, 1930).

Simić (1933), on the basis of comparative morphology and of cross-infection experiments, concluded that *T. parva* of the rat was identical with *T. intestinalis* (= *T. hominis*) of man. Morénas (1938) identified *T. parva* as well as the other species mentioned by Wenrich (1924), but most of his figures of this species apparently are of *T. hominis*. In 1946, Wenrich, recognizing that the species he had called *T. parva* in 1924 and 1930 did not match Alexeieff's figure of that species, and consequently was left without a name, proposed the name *T. wenyoni* for it. This species is more fully described by Wenrich and Nie (1949). Morénas (1938) described two new species, neither of which we have been able to identify in our material.

The new species described below was originally obtained from *Microtus pennsylvanicus*, and therefore the name *T. microti* is proposed for it. A preliminary description without a name has been published in abstract form (Wenrich and Saxe, 1948). As mentioned in our abstract, *T. microti* closely resembles *T. (Pentatrachomonas) hominis*. Throughout the present paper, in our use of the name *T. hominis*, we shall have in mind the prevalent and characteristic form with five anterior flagella.

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MATERIALS AND METHODS

A vole, (*Microtus pennsylvanicus*), caught in a trap by Dr. R. G. Schmieder in July, 1947, provided us with our original material. Contents of the caecum showed small numbers of several kinds of flagellates. Smears were fixed and stained, and some caecal material was fed to three parasite-free rats (*Rattus norvegicus*). In these hosts good populations of a small trichomonad developed. From these rats material was obtained for cultures, for further feeding experiments and for additional fixed and stained slides.

Fixatives commonly used were Schaudinn's and Hollande's fluids. Stains were mostly Heidenhain's hematoxylin and a modified Bodian silver-protein (protargol) impregnation method. Silver impregnation was usually preceded by a bleach as recommended by Honigberg (1947). From cultures some smears were fixed with osmic fumes, dried, and stained with Giemsa's stain.

A considerable collection of stained slides from other rodents, especially rats and mice, was available for comparative studies.

DESCRIPTION

As indicated in the published abstract, *T. microti* resembles *T. hominis* in general appearance, but differs in certain respects. Some of these differences are quantitative and others qualitative. The quantitative differences are subtle and can be recognized mainly by careful comparison of populations rather than individuals. The qualitative differences are distinct but require properly prepared material for their recognition.

T. microti is somewhat smaller than *T. hominis*, the length measured on stained slides ranging from 4 to 9 microns with averages between 5.4 and 6.8 microns. For example, on one slide 100 individuals of *T. microti* ranged from 4 to 8 microns in length, averaging 5.7 microns; while 100 individuals of *T. hominis* from a similar slide gave a range in length from 5 to 9 microns, with an average of 7.1 microns. These measurements were made with an ocular micrometer and did not include the protruding portion of the axostyle; both were made on slides fixed with Schaudinn's fluid. Other sets of measurements gave comparable figures.

In general, normal specimens of *T. microti* seem more plump and to have a somewhat greater dorsal convexity than *T. hominis*; members of the latter species usually appear more slender (Fig. 19).

Characteristically, *T. microti* has four anterior free flagella while five seems to be the rule for *T. hominis* (Wenrich, 1944a, 1944b, Kirby, 1945). They are about as long as the body and may adhere proximally for some distance before separating from each other (Figs. 1, 2, 4, 6, 11, 12, 13, 14, 15). In protargol preparations a small knob is noticeable at the tips (Figs. 11, 12, 13, etc.).

Only one blepharoplast has been seen at the base of the flagella whereas the five-flagellated *T. hominis* has a small blepharoplast for its "independent" flagellum, in addition to the larger one for the other flagella (Fig. 10). Commonly the blepharoplast does not impregnate with silver. As in trichomonads generally, the blepharoplast is also the point of origin for the undulating membrane with its accompanying fibrillar margin and basal costa (chromatic basal rod), and for the parabasal apparatus and the axostyle. Likewise associated with it is the pelta to be described presently.

The undulating membrane is of medium height with three to five "humps." It commonly follows a more sharply spiral course posteriorly in comparison with that of *T. hominis*, and therefore may not reach the posterior end of the body (Figs. 1, 2, 4, 11, 12, 15). Figure 16 of a posterior polar view shows the costa and undulating membrane making the spiral through one-half the body circumference.

As in many other species of *Trichomonas*, the thickened margin of the undulating membrane is made up of the posterior flagellum which becomes the trailing flagellum beyond the end of the membrane, and an accessory filament which ends at the posterior end of the membrane. In most preparations the two filaments are too close together to be separately recognized, although their presence is indicated by the thickness of the outer margin which is much greater than that of the trailing flagellum. This greater thickness is more noticeable in the protargol preparations (Figs. 11–18). The duality of the margin of the undulating membrane is more commonly revealed in dried smears stained with Giemsa's stain, but can be seen in wet-fixed, iron alum-hematoxylin stained slides (Figs. 3, 4, 5), and also in protargol preparations which may show the trailing flagellum separating from the accessory filament at a point short of the posterior termination of the undulating membrane (Figs. 14, 18). On protargol slides the two filaments are usually less distinct than they are in *T. hominis*.

The costa is well-developed and in silver-protein preparations appears to be somewhat thicker (Figs. 11–18) than that of *T. hominis* (Fig. 19). In Heidenhain's preparations the middle portion is somewhat stouter than the terminal parts (e.g., Fig. 5). Paracostal (supracostal) granules are sometimes seen (Figs. 4, 5) as in *T. hominis* (Fig. 10).

The parabasal apparatus resembles that of *T. hominis*. In protargol preparations Kirby (1945) identified a small argentophile ellipsoid body against the right anterolateral surface of the nucleus as the parabasal body of *T. hominis*. Occasionally he saw a thin strand extending forward from this to the blepharoplast, and suggested the possibility of a fibril extending posteriorly. Wenrich (1944a, 1944b) noted the presence of a fine fibril or a row of fine granules in *T. hominis* in the position commonly occupied by the parabasal apparatus in slides fixed with various fixatives and stained with iron-hematoxylin. The junior author discovered that the parabasal apparatus of *T. hominis* as seen on silver-protein preparations, does actually include a posteriorly directed strand from the small round body (Fig. 19) as well as a forward one. *T. microti* has a similar parabasal that is revealed by the protargol technique. Appearances vary from a simple granule (Figs. 14, 15, 16) to such a granule plus a fibril (Fig. 17) or plus a Y-shaped strand with the granule at or near the bifurcation (Figs. 11, 13, 18). In *T. hominis*, Nie and Saxe (ms) have found Y-shaped parabasal bodies in favorable protargol material. A somewhat similar biramous parabasal, usually without the granule was found in *T. wenyoni* by Nie (Wenrich and Nie, 1949).

On protargol slides, a pelta, resembling that described for *T. hominis* by Kirby (1945), has been found at the extreme anterior end of *T. microti*. Its appearance varies somewhat on different slides. Most of our protargol material was prepared after Hollande's fixation and in this material, bleached or unbleached, a side view shows the pelta as a wedge-shaped structure with its apex directed dorsally; the ventral portion appears to be connected with the remainder by a narrow region of somewhat reduced density (Figs. 13, 14). In dorsal view (Fig. 15) the pelta may

show as a crescent, and in a posterior view (Fig. 16) it appears to be somewhat rectangular. Protargol slides prepared without bleaching after Schaudinn's or Petrunkevitch's fixation show an area of deeper impregnation extending posteriorly along the ventral border of the axostyle for some distance (Figs. 17, 18). This impregnated extension along the axostyle may be an additional structure, although possibly it is a part of the pelta. If the latter were the case, it would make the pelta more like that of *T. hominis* (Fig. 19). Compared with Hollande's fixed material the pelta after Schaudinn's or Petrunkevitch's fixation shows a greater lateral extension and appears in dorsal view as a prominent crescent at right angles to the costa (Fig. 17).

The axostyle of *T. microti* is of the same general type as that of *T. hominis*, being widest anteriorly, but lacking endoaxostylar granules. It tapers gradually towards the posterior end, with a more abrupt tapering outside the body than is seen in *T. hominis*. There is no chromatic ring at the point of emergence (contrast with *T. minuta*, Fig. 8). On protargol slides, the region outside the body is more heavily impregnated than the more anterior portions (Figs. 11–15, 17). This feature seems to be more pronounced than in *T. hominis*.

T. microti was cultured in modified Ringer's solution containing Loeffler's dried blood serum, both at 32–33° C and at room temperature. Dry Loeffler's serum powder, or powdered gastric mucin, and water were added from time to time to maintain the cultures. On Loeffler's alone, maximum survival in any one tube was 62 days at 32–33° C and 82 days at room temperature, while those kept at 32–33° and fed powdered gastric mucin survived much longer, up to 278 days in one case.

The living animals, as seen in culture media, display a type of swimming which is more erratic, less vigorous and less progressive than that of *T. hominis*.

As already noted, *T. microti* from the original vole was transferred by feeding to rats. One rat was still infected when sacrificed a year later. Two young guinea-pigs were infected experimentally. Two young hamsters (*Mesocricetus auratus*) from an isolated colony, which, except for *Giardia muris*, was free from associated protozoa, were also infected experimentally. Examination of a series of slides from other rodents showed infections with this species in the source and other individuals of *Microtus pennsylvanicus*, in one *Peromyscus leucopus* (Fig. 7), one wild Norway rat (Figs. 5, 6), and several golden hamsters. Many laboratory rats contained *T. hominis* which has also been found in the hamster. A single attempt to infect a human volunteer with a culture of *T. microti* was unsuccessful.

DISCUSSION

In addition to the species of *Trichomonas* described from rodents belonging to the MURIDAE (as used by Ellerman, 1941) that were mentioned in the introduction, the following have been described as new: *T. mystromyis* Fantham, (1925) from *Mystromys albicaudatus*, *T. myoxi* Galli-Valerio (1927), from *Myoxus quercinus*, *T. guiarti* Morénas (1938) from *Epimys norvegicus* and *Ditrichomonas lavieri* Morénas (1938) from *Pitymys subterraneus*. Now we add *T. microti* to the list. Unnamed species have been reported from a number of other members of the MURIDAE, especially by Pearse (1929).

Fantham's description of *T. mystromyis* indicates that this is very similar to if not identical with *T. muris*. Galli-Valerio's *T. myoxi* was not fully enough described

to compare readily with other species. It is apparently different from *T. muris*, although larger than *T. microti*, and is said to have two anterior free flagella.

We have not seen trichomonads conforming to the descriptions of the two new species named by Morénas (1938). Also, it would appear that this author has misidentified some of the other species mentioned in the introduction. In his figure VI, drawings 1 to 5 are labelled "*T. parva*," but all show three anterior flagella and all have the full-length undulating membrane of *T. hominis*, except drawing 3, which shows a short, sharply spiral membrane more like that of *T. wenyoni* (cf. Fig. 9). Drawing 5 represents an individual too degenerate to identify. On the other hand, his drawings 6 to 8 of this figure are apparently of *T. minuta*, as he has indicated, although they fail to show the costa and cytoplasmic granules of this species. *T. guarti*, illustrated in his figure VII, drawings 3 to 5, is said to be 5.2 to 12.4 microns long, and is therefore larger than *T. microti*. It has a prominent cytostome, while that of *T. microti* is obscure, and the number of anterior flagella is said to be three (exceptionally 4). The costa is obscure (shown only in drawing 3) and no axostyle was found. Drawings 3 and 4 show several rows of cytoplasmic granules. These characters are unlike those of any other species in the MURIDAE known to us, unless they represent small degenerate specimens of *T. muris*. Likewise, the characters of *Ditrichomonas lavieri*, illustrated in his figure VII, drawings 6 to 8, with only two anterior flagella (none on drawing 8), axostyle and cytostome generally invisible, a peculiar granular nucleus, and a range of length from 8.6 to 13.7 microns, indicate a species different from all others heretofore described, and certainly different from *T. microti*. It is possible that these, too, are degenerate representatives of some other species.

Trichomonads are common in rodents, especially the MURIDAE and other ground-dwelling kinds. Just how many species there are is not known since a relatively small number of the described species of MURIDAE has been thoroughly examined for intestinal protozoa. One species of host may harbor several species of trichomonad (Wenrich, 1924, 1930, 1946; Crouch, 1933), and the same species of trichomonad may be found in a number of different kinds of host. For example, Kirby and Honigberg (1949) using their own observations and a review of the literature, report *T. muris* or *T. muris*-like forms as occurring in four species of *Peromyscus*, two species of *Microtus*, and one species each in *Dipodomys*, *Neotoma*, *Arvicola*, *Apodemus* and *Cricetus* (hamster), besides the black and gray rats and the house mouse, among the MURIDAE, and also in four species of *Citellus* (ground squirrels) and one species each of *Hystrix* (Porcupine), *Marmota* (groundhog) and *Ondatra* (muskrat). In addition, Tanabe and Okinami (1940) reported one (also a *Pentatrichomonas*) from *Eutamias asiaticus*. Our *T. microti* has shown itself capable of living not only in *Microtus pennsylvanicus*, but also in *Peromyscus leucopus*, *Rattus norvegicus*, *Mesocricetus auratus* and *Cavia porcella*. It will require extensive transfaunation experiments to determine host-parasite relationships between the various supposed species of *Trichomonas* and the different kinds of hosts. Meanwhile, it is best to recognize species on a morphological basis rather than upon original host relationships.

The finding of a four-flagellated *Trichomonas* in rats and mice which also harbor the five-flagellated *T. hominis* raises some interesting questions. Gabaldon (1930), Dobell (1934) and the authors of some textbooks support the idea that there are

three races of intestinal trichomonad in man, one with three, one with four and the other with five anterior flagella as the characteristic number. Dobell believes that the number is not constant in any of these races. In our opinion, the existence of three races with different numbers of flagella has not been satisfactorily demonstrated. However, variations in flagellar number have frequently been reported, and were even reported for clone cultures by Wenrich (1944a and b), but five seemed to be the more common number. It is possible that variability of the number of anterior flagella is characteristic of *T. hominis*, whereas most other species have a relatively constant number.

If murine rodents, which harbor *T. hominis*, can also contain a closely related species with only four anterior flagella, one could argue that man may also harbor such a species or variety. However, so far as we are aware, no one has described a race from man with the characteristics of *T. microti*, and the one man who swallowed a culture of the latter did not become infected. Obviously the intestinal trichomonads of man require further study.

SUMMARY

1. *Trichomonas microti*, n. sp., was originally obtained from a vole, *Microtus pennsylvanicus*. When caecal material of this host was fed to parasite-free rats, good populations of *T. microti* developed. Subsequently other rats, two young guinea-pigs and two trichomonad-free hamsters were infected. Study of stained slides revealed this species in the original and other voles, in one *Peromyscus leucopus*, one wild *Rattus norvegicus* and several *Mesocricetus auratus*. A human volunteer who swallowed a culture did not become infected. Individual cultures remained positive for as long as 278 days at 32–33° C and for 82 days at room temperature.

2. *T. microti* is slightly smaller, and more rotund than *T. hominis*. There are four anterior flagella instead of five as seen in *T. hominis*, and only one blepharoplast. The undulating membrane is similar to that of *T. hominis* but characteristically follows a more markedly spiral course posteriorly. Its margin is thicker than that of *T. hominis* on protargol slides and is made up of the posterior flagellum, which continues as a trailing flagellum, and an accessory filament which ends at the posterior end of the membrane. The costa is like that of *T. hominis*, but appears thicker in silver-protein preparations. Supracostal granules are sometimes seen on Heidenhain-stained slides.

3. The parabasal apparatus resembles that of *T. hominis*, consisting of a parabasal strand which may be Y-shaped, and a small round or oval body at midpoint or near the bifurcation of the strand.

4. The pelta appears to be more rectangular than that of *T. hominis* and varies in appearance on different slides. On Hollande's fixed-protargol slides it is wedge-shaped in side view; on Schaudinn's fixed slides, the anterior ventral margin of the axostyle is also impregnated, making this region appear to be an extension of the pelta.

5. The axostyle is of the same type as that of *T. hominis*, but appears to be wider, and outside the body tapers more abruptly to the sharp posterior tip. The terminal portion tends to impregnate more strongly with silver than does that of *T. hominis*.

6. The nucleus is similar to that of *T. hominis*. Perinuclear cytoplasmic granules are sometimes seen.

7. The cytostome is usually inconspicuous. The cytoplasm often contains bacteria and other food bodies.

8. In swimming, *T. microti* is more erratic, less vigorous, and less progressive than *T. hominis*.

9. The finding of a four-flagellated species of *Trichomonas* in murine rodents which also harbor the five-flagellated *T. hominis* raises questions as to the existence of similar forms in man. Apparently no one has described from man a form with all the characters of *T. microti*.

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EXPLANATION OF FIGURES

All drawings were made with the aid of a camera lucida at an initial magnification of X 4000. They have been reduced about $\frac{3}{4}$ in printing. Figures 1-10 are from slides fixed with Schaudinn's fluid plus 5% of acetic acid and stained with iron alum-hematoxylin. Figs. 11-19 are from slides treated with a modified Bodian's silver-protein technique. Figs. 11-16 and 19 are from Hollande's-fixed and bleached slides. Fig. 17 is from a Petrunkevitch's-fixed, and fig. 18 is from a Schaudinn's-fixed slide; these slides were not bleached.

Figs. 1-7, *Trichomonas microti*.

FIG. 1. From original host vole, showing characteristic 4 ant. flagella, sharply spiral undulating membrane, etc.

FIG. 2. From experimentally infected rat. Note relatively broad axostyle.

FIG. 3. From experimentally infected rat. Base of undulating membrane deeply stained.

FIG. 4. From experimentally infected rat. Supracostal granules shown.

FIG. 5. From wild Norway rat. Supracostal granules well shown.

FIG. 6. From wild Norway rat. Perinuclear granules shown.

FIG. 7. From *Peromyscus leucopus*. Note food vacuoles.

FIG. 8. *T. minuta*, same slide as fig. 7. Note 3 ant. flagella, more hyaline axostyle with chromatic ring at point of emergence.

FIG. 9. *T. wenyoni*, same slide as figures 5 and 6. Note 3 ant. flagella, poorly developed undulating memb., no visible costa, numerous cytoplasmic granules anteriorly.

FIG. 10. *T. hominis*, from white rat. Note larger size, 5 longer ant. flagella, with independent flagellum from a separate blepharoplast.

Figs. 11-18. *T. microti*, all from experimentally infected rats.

FIG. 11. Left dorsal view. Note pelta and Y-shaped parabasal.

FIG. 12. Left lateral view. Parabasal almost hidden by costa and undulating membrane.

FIG. 13. Right lateral view. Y-shaped parabasal, with granule.

FIG. 14. Pelta seems to be displaced to right giving more of a face view; granule of parabasal shown.

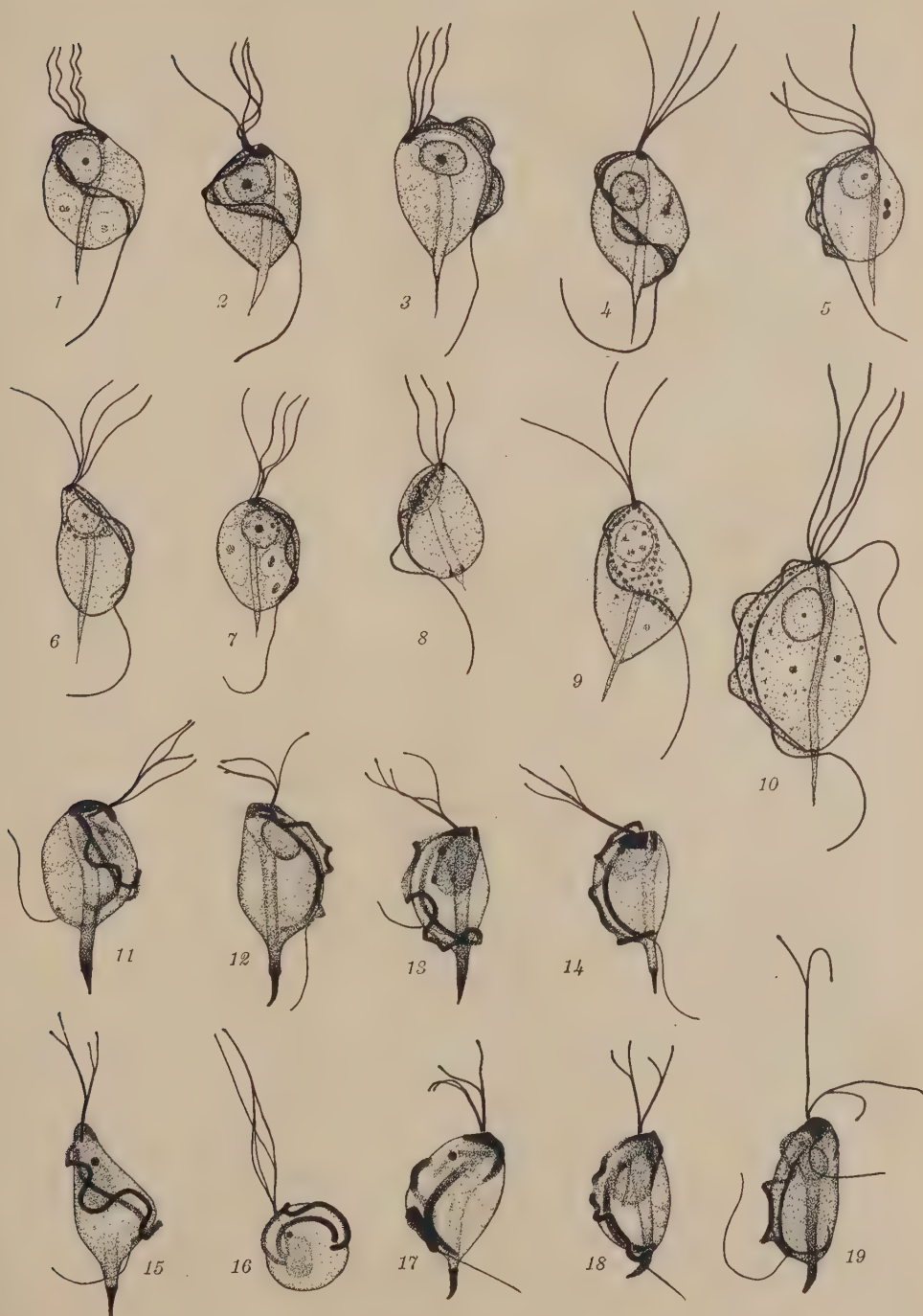
FIG. 15. Dorsal view. Pelta appears as a crescent.

FIG. 16. Posterior polar view. Nucleus and axostyle omitted for greater clarity. Note rectangular pelta; costa and undulating membrane half way circling the body; parabasal granule showing.

FIG. 17. Right ventro-lateral view. Rod-shaped parabasal; heavy impregnation of ant. ventral border of axostyle, apparently as a prolongation of the pelta.

FIG. 18. Right lateral view. Parabasal biramous; an apparent ventral elongation of the pelta.

FIG. 19. *T. hominis*, human source. Note more slender body; 5 ant. flagella; differently shaped pelta; more slender costa and margin of the undulating membrane; parabasal is a strand with median granule.



TWO NEW FLEAS OF THE FAMILY TUNGIDAE*

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Among the fleas received for study from the Chicago Natural History Museum are two new species of the family TUNGIDAE, subfamily HECTOPSYLLINAE. As is so often the case with the members of this family, only the female is known, and it is remarkably adapted for a tick-like existence.

Hectopsylla knighti n. sp.

Diagnosis—Agrees with *H. psittaci* Frauenfeld, 1860, the only other species of the genus known to parasitize birds, in lacking a distinct process on the metepimere. This process is prominent in the mammalian *Hectopsylla* as a caudad-directed stout sclerotization at the upper angle of the posterior margin, and resembles the postoral and prosternal processes. Separated from *H. psittaci* in that the last tarsal segment of the new species possesses but 5 pairs of lateral plantar bristles, not 7 or 8 pairs, and in that the tarsal claws lack a prominent basal tooth.

Description. *Head* (fig 1)—Dorsal margin fairly evenly rounded; anterior margin almost straight; ventral margin somewhat sinuate; with a definite postoral process (*PO.P.*). With but 2 preantennal bristles: a large one in front of eye and a more dorsal small one between the eye and the larger bristle. Eye slightly reduced and somewhat excised ventrally. Genal lobe extending beyond apex of antenna, the angle here well sclerotized and probably serving as an additional structure whereby the flea maintains its hold on the host. Maxillary lobe (*MX.*) apically broad and subrounded; about twice as long as broad. Maxillary palpi long and narrow, reaching to apical fourth of forecoxae. Prementum of labium apparently well developed and somewhat sclerotized, about twice length of maxillary lobe; rest of labium feebly sclerotized and mainly inapparent. Stiletos extremely well developed (fig. 2), being very long, about thrice length of forecoxae and stouter than maxillary palpi. Bristles of second antennal segment much shorter than club. Postantennal region with about 6 thin, scattered bristles, one near midpoint of caudal margin of head, very long.

Thorax—Pronotum with 2 rows of bristles, but second row reduced to but 1 median bristle, longer than those of first row. Mesonotum with 1 subventral bristle and 2 or 3 tiny hairs. Metanotum extremely narrow and heavily sclerotized, apparently devoid of bristles. Prosternum well developed, about twice as long as broad; with a distinct lateral process (*PS.P.*) at dorso-caudal angle. Mesosternum not divided into episternum and epimere; with 1 (or 2?) bristle(s) in anterodorsal angle; with a lateral process (*MS.P.*) near spiracle. Lateral metanotal area (*LM.*) narrow, but distinct; inclined in a plane directed toward metepimeral spiracle. Pleural arch (*PL.A.*) at junction of metanotal ridge and pleural ridge strongly convex, fairly well developed. Metepisternum (*MTS.*) with a long dorsal bristle. Metepimere (*MTM.*) more than twice height of metepisternum; with dorsal margin straight, longer than ventral margin; caudal margin correspondingly sinuate; with a long bristle below spiracle.

Legs—Forecoxa with 7 or 8 lateral bristles. Mesocoxa with about 4 anteromarginal bristles. Metacoxa with anterior margin with about 20 small thin bristles on proximal 2/3s, some of these much longer than others. Femora with a subapical ventromarginal bristle. In addition, mesofemur with about 2 submedian mesal bristles and metafemur with a mesal row of about 6 small thin median bristles. Most of dorsolateral tibial bristles paired. Protarsus with none of apical bristles extending distad of following segment. Mesotarsus with apical bristle of third segment extending beyond apex of fourth. Metatarsus (fig. 5) with first segment bearing an anterior bristle and a posterior apical bristle which extend far distad of third segment; second segment with 3 apical bristles extending to or beyond apex of fifth segment. Distal segment of tarsi with 5 lateral plantar bristles and a long dorsal pair inserted at level of penultimate pair of lateral bristles. Tarsal claw with but a rudiment of a basal swelling. Measurements of tibiae and segments of tarsi (petiolate base deleted) shown in microns:

Leg	Tibia	Tarsal Segments				
		1	2	3	4	5
Pro-	165	44	47	36	33	74
Meso-	236	83	71	55	50	94
Meta-	330	179	105	83	69	94

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Abdomen—Typical sterna without bristles. First tergum (fig. 1, *1T.*) with 2 bristles on each side; others usually with none, occasionally (second and seventh terga) with one small thin bristle. Antepygidal bristles absent.

Modified Abdominal Segments—Seventh sternum (fig. 4, *7S.*) simple, anterior and posterior margins similar; lacking bristles. Eighth tergum (*8T.*) very large, extending almost to level of ventral margin of seventh sternum; bearing on ventral three-fifths three submarginal rows of bristles, of which 2 are mesal and one lateral (the most anterior row mesal, the most ventral bristles and some of the caudal group lateral); the total number of bristles variable, but approximately 40. Anal stylet absent. Sensilium (*SN.*) with 8 or 9 sensory pits on a side and posterior third with about 15–20 bristles. Proctiger weakly sclerotized, with only one lobe apparent and that with about ten small, thin bristles. Spermatheca (*SP.* and fig. 3) with tail not differentiated from head; body fairly regularly oval; but with apex slightly directed downwards; about 4 times as long as broad and about twice as long as tail, which is delimited by a sharp up-turn.

Types—Female holotype *ex a* swift (attached to head). Mexico: Michoacan, Municipality of Tancitaro, elev. 6,000 ft., coll. K. L. Knight for the Third Hoogstraal Mexican Expedition, July 1940. Deposited in the Chicago Natural History Museum. A female paratype with same data in the collection of the senior author.

Comment—This species is named for the collector, Kenneth L. Knight, who has contributed much to the study of medical entomology.

Rhynchopsyllus megastigmata n. sp.

Diagnosis—Immediately separable from *R. pulex* Haller, 1880, by the huge spiracles. The diameter of the spiracle on the second tergum of the new species is almost equal to that of the longer axis of the eye, and is much greater than the length of the penultimate segment of the maxillary palpus. In *R. pulex*, the diameter of this spiracle is only $\frac{2}{3}$ that of the eye and shorter than the length of the penultimate segment of the maxillary palpus. The eighth tergum of the new species bears 3 rows of bristles, 2 of which are mesal and 1 lateral, the lateral bristles inserted almost in a line with those of the posterior mesal row, but thinner and more widely spaced. In *R. pulex* there are but two rows of bristles on the eighth tergum, one mesal and one lateral; those of lateral row also posteriormost but broad, evenly and regularly spaced.

Description. Head (fig. 6)—Dorsal margin evenly rounded; anterior margin almost straight; ventral margin somewhat sinuate, terminating in a conspicuous postoral process (*P.O.P.*). With but one preantennal bristle and that anterior to eye. Eye slightly reduced, longer than broad, and somewhat excised ventrally. Genal process conspicuous, basally broad, apically acuminate; probably serving as an additional holdfast. Acuminate apex of maxillary lobe extending to apex of second segment of maxillary palpus. Stilettoes very long and broad, deeply serrate; extending far beyond apex of forecoxae. Labium and labial palpi very weakly sclerotized. Bristles of second antennal segment much shorter than club. Postantennal region with 6 or more thin bristles in 2 irregular rows.

Thorax—Pronotum with one row of bristles. Mesonotum with one or perhaps two median bristles and tiny mesal bristle near midpoint of anterior margin. Metanotum with about two dorsomarginal bristles. Thoracic nota dorsally heavily sclerotized. Prosternum about twice as long as broad; with a conspicuous subacuminate lateral process (*P.S.P.*). Mesosternum (*MS.*) not divided into episternum and epimere; with one median subdorsal bristle. Lateral metanotal area (*L.M.*) small, ventrally constricted. Pleural arch (*PL.A.*) strongly convex; fairly well developed. Metepisternum (*MTS.*) with a long dorsal bristle. Metepimere (*MTM.*) well developed, about thrice height of metepisternum; dorsal margin somewhat convex; with a row of about four bristles. Spiracle of metepimere smaller than those of abdominal terga (e.g. *2S.*).

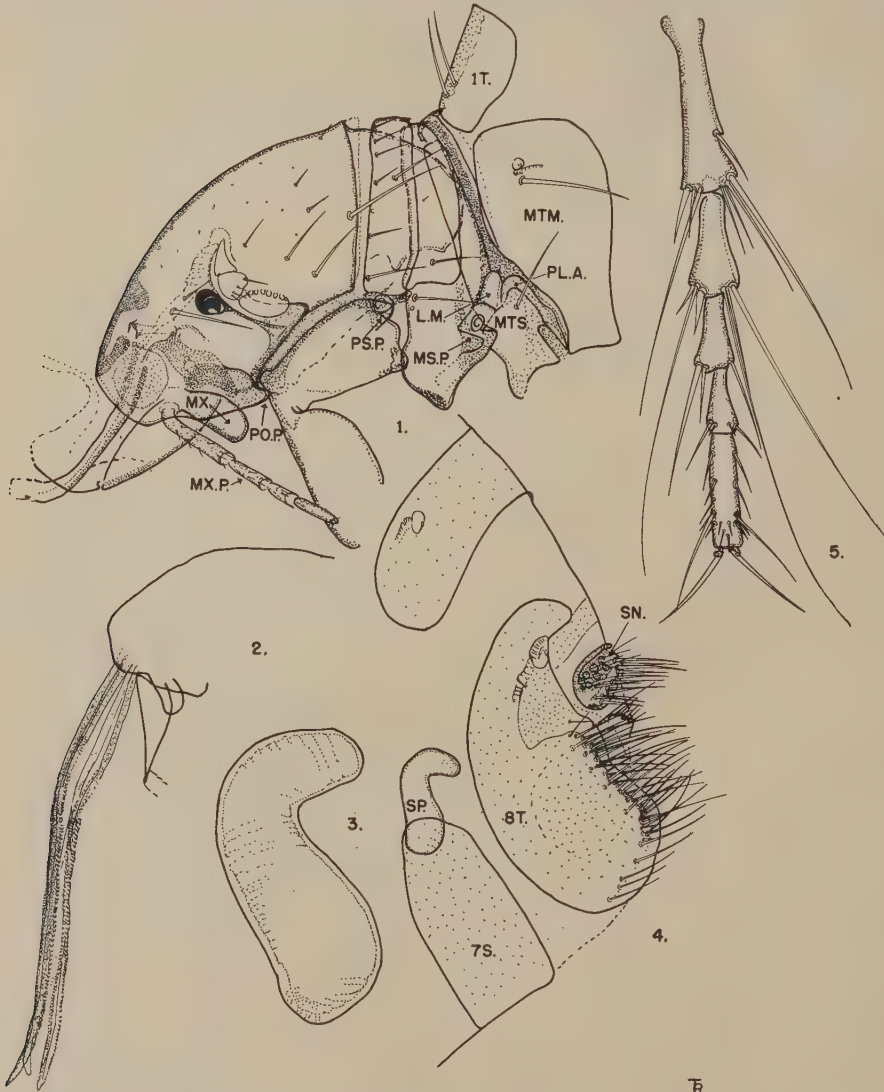
Legs—Forecoxa with about 10 scattered lateral bristles. Mesocoxa and metacoxa with a group of lateral bristles near antero-ventral angle. In addition with a group of anteromarginal mesal bristles on metacoxa, especially on upper half. Femora with a subapical ventromarginal bristle. Metafemur with a mesal row of about 5 or 6 bristles.

Abdomen—Typical sterna without bristles. First tergum (fig. 7, *1T.*) with one or two bristles on a side. Second tergum with 3 bristles on each side, others with none or one. Antepygidal bristles absent.

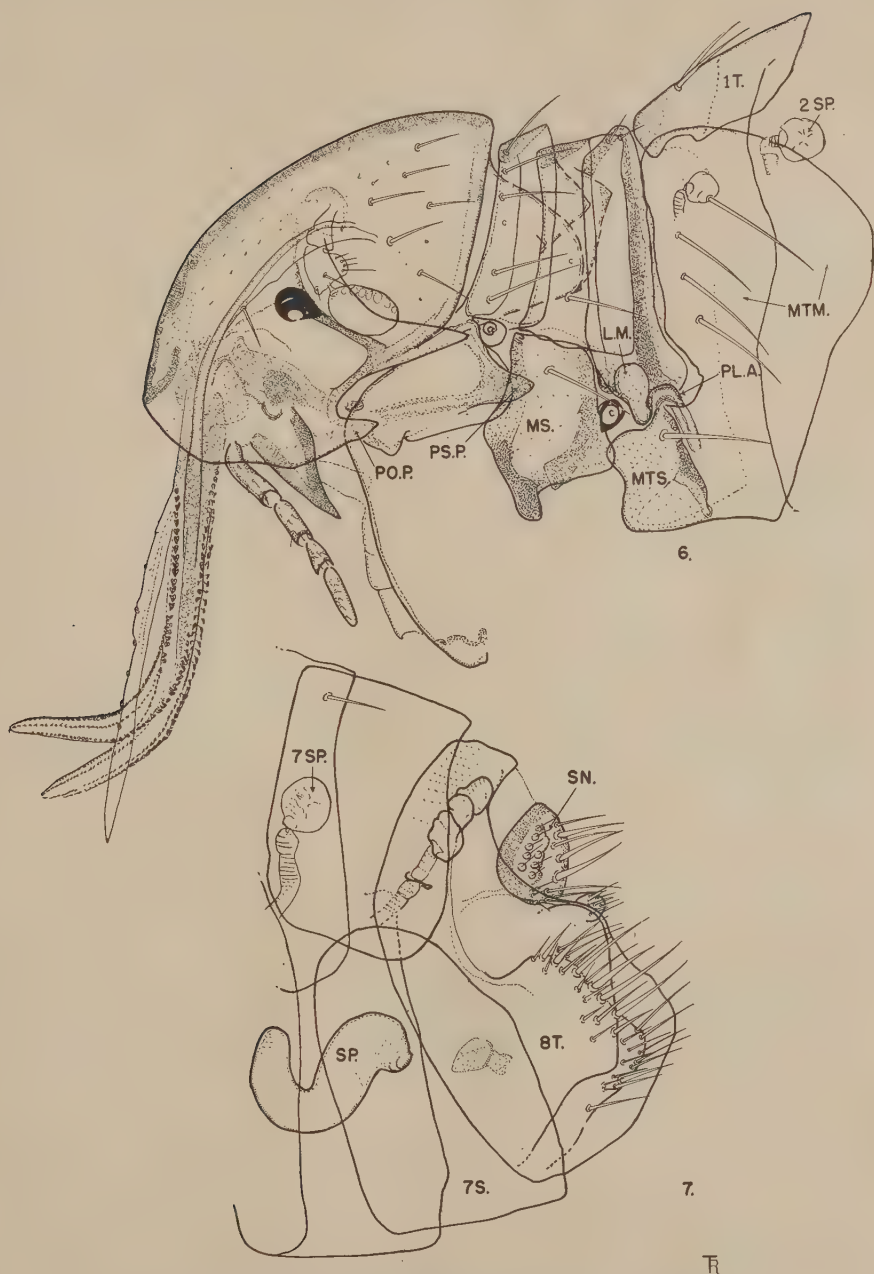
Modified Abdominal Segments—Seventh sternum (fig. 7, *7S.*) devoid of bristles, virtually unmodified. Eighth tergum (*8T.*) very large, extending almost to level of ventral margin of seventh sternum; bearing on ventral three-fifths four submarginal rows of bristles, of which 3 are mesal and one lateral (the most anterior two rows mesal, the others contiguous); with about

eight fairly long thin bristles in lateral row, of which the lowest four are close together; with three or four short bristles in first mesal row, about 11 in second, including four subventral thinner ones; about nine in third row, most fairly stout. Anal stylet absent. Sensilium (SN.) with eight sensory pits on a side and with about five relatively stout bristles near caudal margin and about five or six small ones in ventrocaudal area. Proctiger with only one lobe apparent and that with about ten small thin bristles. Spermatheca (SP.) with tail not differentiated from head but strongly upturned, the organ sigmate.

Types.—Female holotype *ex Tadarida brasiliensis*. Peru: Puno, Santo Domingo, coll. C. C. Sanborn for the Chicago Natural History Museum, 20 Oct. 1941. Deposited in Chicago Natural History Museum. Paratype female with same data in collection of the senior author.



HECTOPSYLLA KNIGHTI SP. NOV.



RHYNCHOPSYLLUS MEGASTIGMATA SP. NOV.

HYMENOLEPIS RAUSCHI, N. SP., A CESTODE FROM THE RUDDY DUCK*

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A number of cestodes were removed from the intestine of *Oxyura jamaicensis rubida*, the ruddy duck, taken at Buckeye Lake, Ohio, in 1943. The majority of the specimens were readily identified as *Diorchis excentricus* Mayhew, 1925. Four larger specimens, occurring in association with these worms, represent a species hitherto undescribed.

Hymenolepis rauschi n. sp.

(Figs. 1-9)

Diagnosis: Length of strobila about 70 mm; maximum width 5 mm, attained at the posterior end of the strobila. Scolex $156 \times 200 \mu$. Suckers, about 100μ diameter, directed forward, weakly muscled and armed with short, blunt spines. Invaginated rostellum 165μ in length and 49μ in diameter at base. Rostellum provided with a single row of 8 hooks, 46μ in length. Strobila 148μ wide immediately posterior to base of scolex. Genital *Anlagen* appear early. Genital pores unilateral and dextral, occurring in anterior one-fourth of the proglottid. Genital ducts ventral to excretory canals. Muscular cirrus sac averages 639μ in length by 142μ in width. Cirrus sac extends medially beyond poral excretory canal for a distance which approximates one-half its length and generally approaches a point above middle of poral testis. Internal seminal vesicle well developed; external seminal vesicle prominent and maintains a position directly aporal to cirrus sac and extends to middle of proglottid. External seminal vesicle measures 255μ in length by 213μ in width in mature proglottids. Cirrus unarmed, protrusible, and provided with a slender, whip-like stylet. Sacculus accessorius absent. Testes three in number, subspherical, about 241μ in diameter and located in posterior one-half of the proglottid. Testes all lie in same plane between excretory canals. Ovary deeply lobed. Ovary and vitelline gland located in middle of proglottid between the two aporal and the poral testes. Vagina lies posterior and ventral to cirrus sac. Large bulbular seminal receptacle appears dorsal to cirrus sac and lies directly anterior to poral testis. Seminal receptacle attains a maximum size of $170 \times 225 \mu$ in mature proglottids. Uterus extends as a slender, irregular tube transversely across anterior part of proglottid. Uterus passes dorsally to and extends beyond the excretory canals. Uterus develops by lobation and enlargement, and becomes sacculate when gravid. Longitudinal muscle bands numerous and distinct in whole mount; approximately 10μ in diameter. Vas efferentia arise on poral side of the antiporal testes and on the antiporal side of the poral testis. Duct from the most distant antiporal testis passes ventral to median testis and unites with the duct from poral testis, thus forming the vas deferens which then passes, by the shortest distance, to the external seminal vesicle. The point of juncture varies considerably in its location and may be found anterior, posterior, aporal or poral to ovary. Ventral longitudinal excretory canals measure 36μ in diameter; dorsal canals, 15μ in diameter.

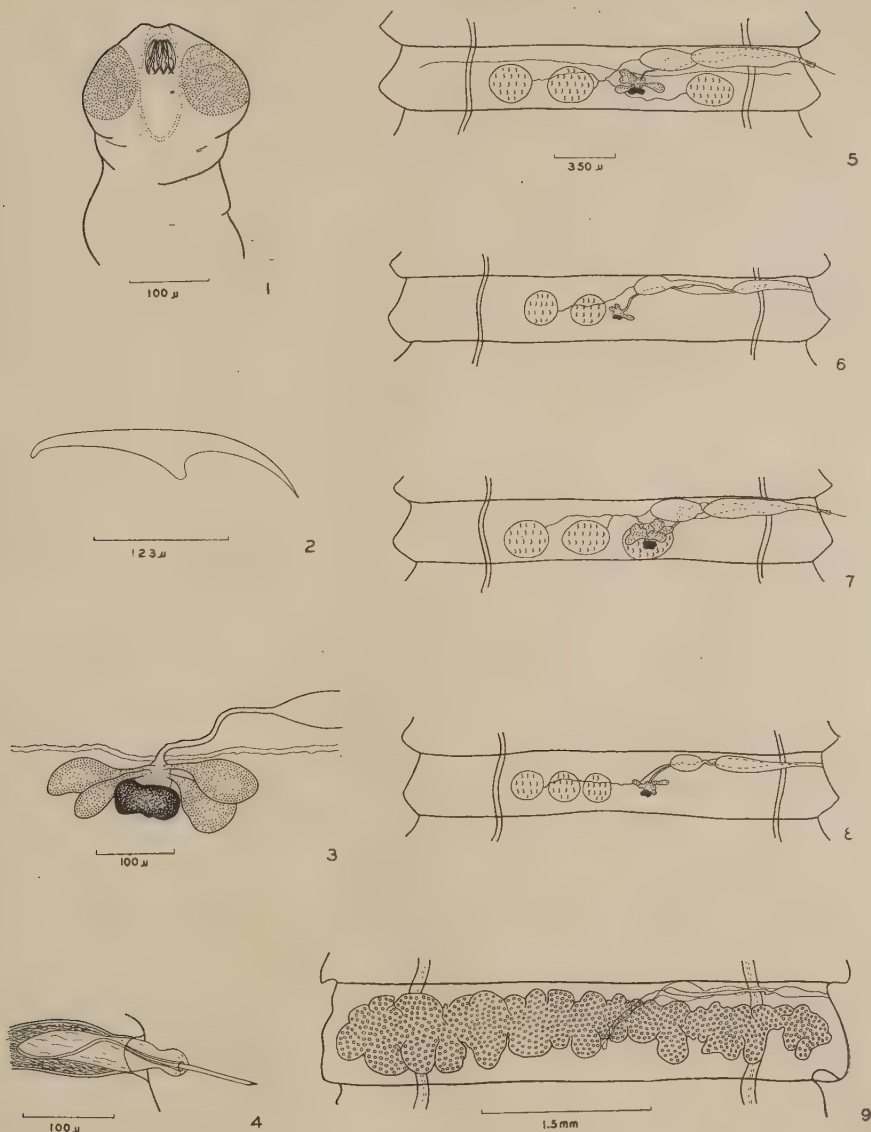
Discussion: An examination of the literature descriptive of the species of *Hymenolepis* found in ANSERIFORMES reveals no knowledge of the existence of a form possessing eight rostellar hooks, armed suckers and a cirrus provided with a stylet. *Hymenolepis neomeggittilis* Hughes, 1940, found in *Spatula clypeatus*, the shoveler duck, (material collected in Egypt and incompletely described by Meggitt, 1927, as *Hymenolepis birmanica*) resembles this species insofar as it possesses eight rostellar hooks and armed suckers; however, no cirrus stylet was observed. The hooks are considerably smaller (29μ) and are of a different shape, the genital pore is in the posterior margin of the proglottid and strobila has a maximum width of only 1 mm.

The combination of armed suckers and eight rostellar hooks supports the opinion that *Echinocotyle*, either as a genus or subgenus, has no valid status.

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¹ Now with the U. S. Public Health Service, Box 960, Anchorage, Alaska.



EXPLANATION OF PLATE

- FIG. 1. Scolex.
 FIG. 2. Rostellar hook.
 FIG. 3. Relationship of female genitalia (ventral view).
 FIG. 4. Protrusible cirrus with extended stylet.
 FIG. 5. Mature proglottid with typical testicular arrangement (ventral view).
 FIG. 6. Somewhat younger proglottid with irregular number of testes (ventral view).
 FIG. 7. Mature proglottid with irregular testicular arrangement (ventral view).
 FIG. 8. Mature proglottid with irregular testicular arrangement (ventral view).
 FIG. 9. Structure of the developing uterus (ventral view).

It is possible that sucker armature may have been inadvertently overlooked in the descriptions of certain forms by earlier writers and, therefore, the present species was compared to all others of the genus *Hymenolepis* having eight rostellar hooks, a cirrus stylet and lacking a sacculus accessorius. At the present time 15 species have been described which are provided with a cirrus stylet (14 as reported by Brock (1942) and one since described, *Hymenolepis hughesi* Webster, 1947). Of this number, six species have eight rostellar hooks and lack a sacculus accessorius. These were, therefore, further considered for comparison and were found to differ from the new species as follows:

Hymenolepis cyrtoides (Mayhew, 1925), the only other member of this genus recorded from the ruddy duck, differs in size, being considerably smaller ($3\text{--}10 \times 0.4$ mm.). The testicular arrangement is triangular, with the testes occurring in two different planes. The length of the rostellar hooks is greater ($67\text{--}70$ μ), and they have a characteristically different shape.

Hymenolepis flamingo Skrjabin, 1915 (parasitic in PHENICOPTERIFORMES) is of smaller size ($18\text{--}25 \times 1$ mm) and has a definitely smaller cirrus sac (260×110 μ). It has longer rostellar hooks ($57\text{--}62$ μ) of a different shape. (The suckers are reported to be unarmed.)

Hymenolepis macracantha (von Linstow, 1877) (parasitic in ANSERIFORMES) is of smaller size (12×0.3 mm), with very large suckers and rostellar hooks of much greater length ($108\text{--}128$ μ).

Hymenolepis serrata Fuhrmann, 1906 (parasitic in COLUMBIFORMES) is smaller in maximum width (800 μ) and has an armed cirrus. The rostellar hooks are of much greater length (102 μ) and have a distinctly different shape.

Hymenolepis serrata var. *birmanica* Meggitt, 1924 (parasitic in COLUMBIFORMES) differs in the same characters as the species of which this form is a variety. (Suckers in both are reported to be unarmed.)

Hymenolepis venusta (Rosseter, 1897) (parasitic in ANSERIFORMES) has a much larger scolex (675 μ), larger suckers (280 μ), a smaller cirrus sac (370×70 μ) and longer hooks ($51\text{--}54$ μ) which are of a characteristically different shape.

The combination of eight rostellar hooks, armed suckers and the presence of a cirrus stylet, together with diagnostic hook size and shape, an unarmed protrusible cirrus and the absence of a sacculus accessorius, provides an aggregation of characters serving to distinguish this species from all other species of the genus *Hymenolepis* Weinland, 1858. It therefore seems justifiable to designate this species as new.

This species was named in honor of Dr. Robert Rausch, of the United States Public Health Service, Anchorage, Alaska, who so generously made available his collection of cestodes from ANSERIFORMES from which the present study was made.

Thanks are due also to Dr. Banner Bill Morgan, Department of Veterinary Science, University of Wisconsin, for helpful suggestions during the course of this study.

Host: *Oxyura jamaicensis rubida* (Wilson).

Locality: Buckeye Lake, Ohio.

Habitat: Duodenum.

Type: One slide, No. 46475, containing an entire specimen, has been deposited in the Helminthological Collection of the U. S. National Museum.

Variability: A critical study of the strobila of *Hymenolepis rauschi* reveals that, for the most part, the three testes occur in the same general pattern of distribution, i.e., two aporal and one poral to the ovary (fig. 5). The testes are well separated from each other as well as from the female genitalia. However, irregularities in the above described pattern occur in some proglottids at various places along the strobila. A proglottid in the early maturation portion of the strobila has only the two aporal testes present (fig. 6). The third proglottid posterior to this, has all three of the testes located aporally and well beyond the ovary (fig. 8). This is considered to be the most extreme example with regard to testes position and is evidenced by at least two proglottids in the strobila. Others, seen at irregular intervals, have the poral testis situated directly dorsal to the ovary (fig. 7). A range in distribution of the testes is noted from that considered to be typical (fig. 5) to that represented by all three testes aporal to the ovary (fig. 8). There is no uniformity of occurrence of these unusual proglottids in the strobila. Mayhew (1925) reported a somewhat similar type of variability in certain specimens taken from *Aythya marila nearctica* (= *Marila marila*), scaup duck, which he described as *Hymenolepis sacciperium*. The variability exhibited by the species herein described differs from that seen in *H. sacciperium* in that four testes have never been observed in a single proglottid, nor in proglottids having two testes does one occur aporal and the other poral. Three testes have not been found on the poral side in *H. rauschi*. All testes, regardless, of distribution and number, are of uniform size. The examination of a large number of other species from anseriform birds, however, has disclosed variations identical with those described by Mayhew; moreover, in a few cases, one or more of the testes concerned in the variability were extremely reduced in size and

appeared to be nonfunctional. This irregularity is not limited to species of *Hymenolepis* having the testes disposed in a straight line, but is also found in those species which have a triangular testicular arrangement.

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A NEW SPECIES OF THE ACANTHOCEPHALAN GENUS *ARHYTHMORHYNCHUS* FROM SANDPIPERS OF ALASKA

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In the spring of 1949, a survey of the parasites of birds of Alaska, especially in the vicinity of Juneau, was conducted by Robert L. Rausch and Ralph B. Williams. Among the Acanthocephala encountered there were specimens of the genus *Arhythmorhynchus* in two species of sandpipers, *Erolia ptilocnemis couesi* (Ridgway) (the Aleutian sandpiper) and *Erolia alpina pacifica* (Vieillot) (the red-backed sandpiper). This parasite was present in such numbers as to make morphological and taxonomic study profitable. The species is clearly distinct from all other members of the genus and is here described as *Arhythmorhynchus comptus* n. sp.

On the first day of collecting (March 3, 1949), three specimens of *Erolia ptilocnemis couesi* were taken from a large flock. Two of these individuals were heavily infected with *A. comptus*. On the following day, 41 birds were taken from apparently the same flock in connection with various studies in progress. These included 15 of the Aleutian sandpiper and 26 of the red-backed sandpiper. In the 15 individuals of *Erolia ptilocnemis couesi* (the Aleutian sandpiper), 167 worms were found in the 11 infected individuals, with an average of slightly more than 15 worms per infected bird and a minimum of 3 and maximum of 42 worms per bird. Of *E. alpina pacifica* (the red-backed sandpiper), 25 of the 26 birds examined were infected with a total of 204 worms, making an average of only slightly more than 8 worms per infected bird, with individual infections ranging from 1 to 20 worms. The parasites were scattered through the lower half of the small intestine without being restricted to any localized portion of it. In both species of host, some other intestinal worms, chiefly cestodes, were encountered but in small numbers. Even the heavily infected birds were fat and neither the weight nor the general appearance gave evidence that the parasites affected the condition of the host.

Subsequently, other land, shore and water birds of the vicinity were examined in the hopes of locating additional vertebrate hosts for *A. comptus*. Although several other species of ACANTHOCEPHALA were found, only one additional host species and an extension of the seasonal occurrence in this and a previously recognized definitive host have been added to the records mentioned above. On October 29, 1949, R. B. Williams examined a specimen of the surf-bird, *Aphriza virgata* (Gmelin), from the Lynn Canal near the mouth of Eagle river, approximately 30 miles north of Juneau. Two immature specimens of *Arhythmorhynchus comptus* were recovered from the intestine of the surf-bird and from the same vicinity a single large but immature specimen of the same parasite was found in each of two individuals of the Aleutian sandpiper. These constitute the only available records of the occurrence of *A. comptus* in extension of the records upon which the description of the species is based.

S. Yamaguti (1939: 333) described as *Polymorphus eroliae* an acanthocephalan which he discovered in *Erolia alpina sakhalina* (Vieillot) of Sizuoka Prefecture, Japan. Both the illustrations and the description which he gave indicate that the generic assignment was in error, since the features of this species are clearly those of the genus *Arhythmorhynchus*. Consequently, *Polymorphus eroliae* Yamaguti, 1939 becomes *Arhythmorhynchus eroliae* (Yamaguti, 1939). This species has many points of morphology in common with *Arhythmorhynchus comptus*. However, the two species are clearly distinguishable one from the other on the basis of size of the proboscis and number of proboscis hooks.

Taxonomically related birds of the European continent carry a wide representation of acanthocephalan parasites, but in no instance is there any species recorded which even remotely resembles *Arhythmorhynchus comptus*. *A. frassoni*, *A. invaginabilis*, *A. macrourus*, and *A. longicollis* are the only representatives of the genus recorded from northern and central European birds. All but the last one of these have such distinctively different formula of proboscis armature that they could not possibly be confused with *A. comptus*. *A. longirostris* is very imperfectly known. Both Baylis (1922: 426) and Meyer (1931: 14 and 1932: 45) record the species without giving any information on the number and arrangement of the proboscis hooks although both refer to the proboscis as cylindrical or nearly so. On this feature, *A. comptus* is markedly different from *A. longicollis*.

Some light is thrown upon the rather sharp geographical limitations and host relations of *A. comptus* by examination of negative records for birds closely related taxonomically or ecologically to the normal hosts (Table 1). Eleven of 14 species of sandpipers and taxonomically related birds (a total of 41 individuals), collected in Alaska, were all negative for *A. comptus* and specimens of one of its normal hosts, the red-backed sandpiper, from different flocks and localities were likewise negative. Furthermore, previously unpublished observations (Table 1) on the parasites of 169 additional specimens representing 21 species of sandpipers, plovers and related species, from various localities in Canada and the United States were likewise all negative for *A. comptus*. Ordinarily, negative records have relatively little significance, but when they are limited to potential hosts of similar habits, and of fairly uniform taxonomic relationship, they serve as a means of delimiting the geographical and seasonal distribution and host relations of a parasite.

Field collections, by Rausch supplemented by materials collected by Williams, include such a comprehensive sampling of related birds on the North American continent that the results are incorporated as Table 1.

The parasites were killed in Alcohol-Formol-Acetic (A.F.A.) mixture and were shipped immediately to Urbana by air mail in this fluid. Upon arrival they were transferred to alcohol. In life and following preservation, the specimens were of bright orange color. Attempts to bleach with chlorine and other routine bleaching procedures were unsuccessful but when the specimens were brought into 95% alcohol the color leached out completely. An unbleached specimen and the supernatant alcohol were treated with concentrated sulphuric acid, whereupon they turned a greenish blue indicating that the color was probably due to the presence of carotene. In this connection, it is interesting to note that most if not all of the species of ACANTHOCEPHALA which are yellow or orange in life, are found in the intestine of vertebrates which feed upon arthropods.

TABLE 1.

Records of the examination of North American sandpipers, plovers, and related birds for ACANTHOCEPHALA of the genus *Arhythmorhynchus*. *, indicates *A. comptus* found; —, birds examined but no *A. comptus*; 0, no birds of this species examined from the locality

The species are arranged in the sequence of the A. O. U. Check-list, prepared with the co-operation of Dr. Harvey I. Fisher.

	Alaska	United States and/or Canada
<i>Ereunetes pusillus</i> semipalmated sandpiper	—	—
<i>Charadrius wilsonia wilsonia</i> Wilson's plover	0	—
<i>Charadrius vociferus</i> killdeer	0	—
<i>Charadrius hiaticulata semipalmatus</i> semipalmated plover	—	—
<i>Pluvialis dominica</i> golden plover	—	—
<i>Squatarola squatarola</i> black-bellied plover	—	0
<i>Aphriza virgata</i> surf-bird	*	0
<i>Phalaropus minor</i> Woodcock	0	—
<i>Capella gallinago delicata</i> Wilson's snipe	0	—
<i>Numenius americanus</i> long-billed curlew	0	—
<i>Numenius phaeopus variegatus</i> Hudsonian curlew	—	0
<i>Bartramia longicauda</i> upland plover	0	—
<i>Actitis macularia</i> spotted sandpiper	0	—
<i>Tringa solitaria</i> solitary sandpiper	0	—
<i>Heteroscelus incanus</i> wandering tattler	—	0
<i>Totanus melanoleucus</i> greater yellow legs	0	—
<i>Totanus flavipes</i> lesser yellow legs	0	—
<i>Calidris canutus</i> knot	0	—
<i>Erolia ptilocnemus couesi</i> Aleutian sandpiper	*	0
<i>Erolia melanotos</i> pectoral sandpiper	—	—
<i>Erolia bairdii</i> Baird's sandpiper	—	—
<i>Erolia minutilla</i> least sandpiper	—	—
<i>Erolia alpina pacifica</i> red-backed sandpiper	*	—
<i>Limnodromus griseus</i> dowitcher	—	—
<i>Micropalama himantopus</i> stilt sandpiper	—	—
<i>Crocethia alba</i> sanderling	0	—

Arhythmorhynchus comptus n. sp.

(Figs. 1-6)

Description. With the characteristics of the genus *Arhythmorhynchus*. Body (Fig. 3) very long and narrow with a slight inflation near the anterior end. Proboscis short, spindle-shaped. Neck (N) relatively long. Females 40 to 55 mm in length with a maximum diameter of from 0.8 to 1 mm; proboscis 0.43 to 0.48 mm long by 0.25 to 0.40 in width at near middle; neck 0.46 to 0.80 mm long. Proboscis (Fig. 1) armed with 15 or 16 longitudinal rows of 8 or 9 hooks each, those near middle of proboscis usually 0.044 to 0.048 mm long, relatively heavy but frequently with poorly defined roots. Embryos (Fig. 6) within body cavity of females 0.093 to 0.101 mm long by 0.022 to 0.026 mm wide, with a short, rounded polar prolongation at each end.

Males: (Fig. 3) 23 to 32 mm long with maximum diameter of 0.635 to 0.807 mm; proboscis (Fig. 2) slightly smaller than in female, 0.32 to 0.44 mm long by 0.17 to 0.24 mm in maximum width; neck 0.35 to 0.75 mm long. Proboscis armature as in female, except that more of the hooks are about 0.044 mm in length. Two testes (TA, TP) contiguous, each about 1 to 1.5 mm long, located in anterior part of body about 2 or 3 mm behind the inflated, spine covered area (S). Cement glands (C) apparently only two, extremely long and tubular, from 13 to 24 mm in length.

Neck and anterior region of trunk of both sexes covered with a thick, hyaline cuticula. Fore part of trunk (Fig. 3a) somewhat inflated (I) for a distance of about 2.5 mm, often with a slight secondary constriction at about the anterior third. A relatively short spine field 1 to 2.5 mm in length (S) encircles the anterior extremity of the trunk with the anterior spines closely set but progressively more widely dispersed posteriorly. Roots of the body spines more conspicuous in stained mounts than the spines, the latter usually about 0.015 mm long with roots irregularly circular in outline, about 0.015 to 0.020 mm in diameter.

The subcuticular nuclei of the trunk are restricted to a zone in the inflated region of the trunk and are wholly lacking both posterior and anterior to this region. In mature individuals, these nuclei of the subcuticula (Fig. 5, N F) are highly fragmented, rounded bodies from 0.010 to 0.025 mm in diameter, packed so closely that the diameter of each fragment is usually less than the distance between the fragments. The nucleated zone starts at a level near the posterior end of the proboscis receptacle, almost coincident with the posterior limit of the spine field, and extends for a distance of 1.5 to 3 mm, forming an uninterrupted band around the entire circumference of the body. In males of *A. comptus*, the nucleated zone extends back-

ward somewhat beyond the anterior limit of the anterior testis. Both anteriorly and posteriorly the nuclear fragments become more widely dispersed but in a relatively short distance they disappear completely, leaving most of the vast syncytial subcuticula entirely devoid of nuclei. This restricted localization of the subcuticular nuclei is apparently distinctive of the genus *Arhythmorhynchus* (Van Cleave, 1916). In some other genera of ACANTHOCEPHALA, there is no fixed pattern of localization while the sharpest contrast with conditions found in *Arhythmorhynchus* is that encountered in the family NEOECHINORHYNCHIDAE. In the last mentioned family, the giant nuclei of the subcuticula are widely dispersed in a longitudinal linear series with all of them restricted to the sagittal plane of the trunk.

The main trunks of the lacunar system are chiefly lateral in position. Most of the females are so filled with developing eggs that details of the genital tract could not be made out. In a young female which had not become gravid (Fig. 4), the vagina (V) and part of the uterus (U) are clearly shown but the uterine bell and the selective apparatus are obscured by other structures. The double-walled receptacle of the proboscis bears the brain near the middle of its extent. The lemnisci (L, Fig. 3a), irregularly tubular in shape, extend about 1.2 to 1.8 mm posteriorly into the trunk.

Hosts: *Erolia ptilocnemis couesi* (Ridgway), the Aleutian sandpiper, and *Erolia alpina pacifica* (Vieillot), the red-backed sandpiper in the vicinity of Juneau, Alaska, on March 3 and 4, 1949. Additional records from the Aleutian sandpiper and the surf-bird, *Aphriza virgata* (Gmelin), near the mouth of Eagle River, Alaska, October 29, 1949.

Developmental stages: Wholly unknown.

Type material: Holotype male, allotype female and series of paratypes of both sexes in collection of H. J. Van Cleave, Urbana, Illinois and paratypes in collection of Robert L. Rausch, Anchorage, Alaska.

Comparisons: Because of the wide geographical distribution and migratory habits of many of the species of the family SCOLOPACIDAE, to which the sandpipers belong, and likewise because of the similarities in food habits and in ecological relations of water and shore birds, a careful check has been made between *A. comptus* and all other members of this genus. No member of this genus has been reported previously from sandpipers of either Europe or America (Meyer 1932-33).

Arhythmorhynchus eroliae (Yamaguti, 1939) and *Polymorphus capellae* Yamaguti, 1935, both from Japan, are the only two species of ACANTHOCEPHALA previously reported from sandpipers and related birds which are considered in this report. Both of these species are clearly distinct from *A. comptus*. While the last named species has proboscis armature consisting of 15 or 16 longitudinal rows of 8 or 9 hooks each, *A. eroliae* has 18 longitudinal rows of 12 or 13 hooks each and *Polymorphus capellae*, of uncertain generic assignment, has 14 to 16 hooks in each of its 17 or 18 longitudinal rows.

A. comptus is clearly distinct from every other known species of the genus, all of which have conspicuously greater numbers of proboscis hooks. This is particularly true of the numbers of hooks in each longitudinal row. In *A. comptus*, there are but 8 or 9 hooks per longitudinal row while the smallest number recorded for any other species of the genus is 13. Previous studies have shown that individual variability of this magnitude is never found in members of the genus *Arhythmorhynchus*.

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EXPLANATION OF PLATE

Katharine Hill Paul, scientific artist in the department of Zoology, University of Illinois, prepared the drawings and arranged the plate. All drawings were made with the aid of a camera lucida from stained specimens mounted in clarite.

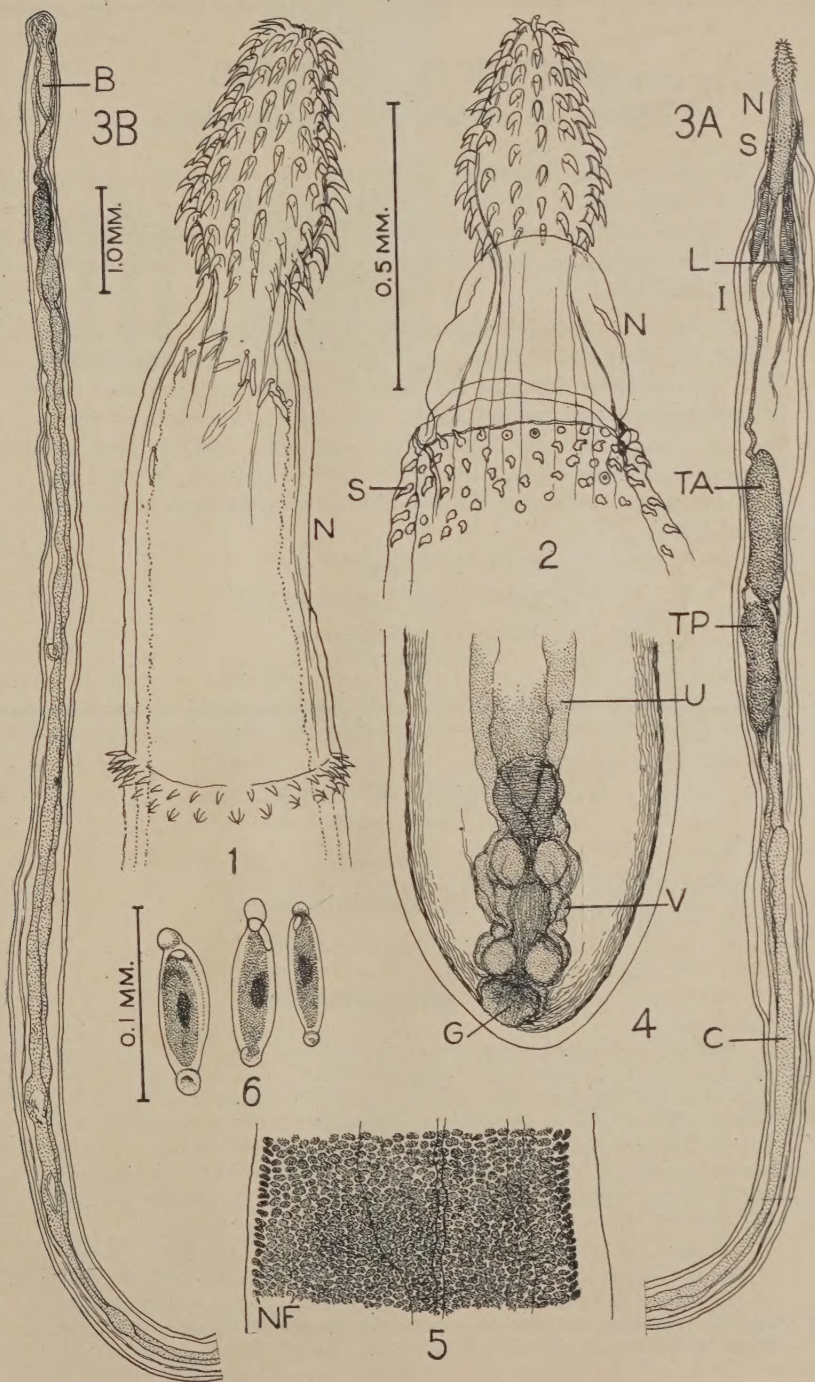
The scale between Figs. 1 and 2 applies to both of these and likewise to Figs. 4 and 5.

SYMBOLS

B—introverted bursa	NF—nuclear fragments
C—cement glands	S—body spines
G—genital pore	TA—anterior testis
I—inflated region of trunk	TP—posterior testis
L—lemniscus	U—uterus
N—neck	V—vagina

Morphology of *Arhythmorhynchus comptus* n. sp.

- FIG. 1. Proboscis, neck and anterior end of trunk of a female.
 FIG. 2. Proboscis, neck and anterior end of trunk of a male.
 FIG. 3. Entire body of holotype male (A) anterior end, (B) posterior end.
 FIG. 4. Posterior extremity of an immature female showing vagina (V) and a portion of the uterus (U).
 FIG. 5. Segment of the trunk of a male in the inflated region showing distribution of fragmented nuclei of the subcuticula.
 FIG. 6. Eggs from the body cavity of a female.



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